日本癌学会及財團法人癌研究会発行

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THE JAPANESE JOURNAL OF CANCER RESEARCH

Founded by K. YAMAGIWA and Continued by M. NAGAYO

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[GANN, Vol. 44, March, 1953]

AMINO ACIDS AND TOXOHORMONE SYNTHESIS. A FIFTH STUDY ON TOXOHORMONE, A CHARACTERISTIC TOXIC SUBSTANCE PRODUCED BY CANCER TISSUE

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INTRODUCTION

Since our first demonstration in 1948 of toxohormone, a characteristic toxic substance produced by all cancer tissues, which is assayable by its depressing effect on liver catalase (1), our knowledge concerning this unique substance has been much extended. We now know that, chemically, toxohormone may be a kind of polypeptide closely associated with the non-heat coagulable protein fraction (2). The mode of its action seems to be connected with the interferance of the utilization of iron, a supply of extra iron being capable of reducing the toxohormone effect (3). Evidence has also been obtained to show that toxohormone may adversely affect the protein metabolism, as indicated by the characteristic involution of thymus (4). This last point strongly suggests that toxohormone may be intimately bound up with the causation of the so-called cancer cachexia.

During the last few years attempts have been made to experimentally modify the toxohormone content of cancer tissue. This line of investigation was suggested from the point of view that by some such means as this it may be possible to learn something of the mode of toxohormone synthesis by cancer tissue. Our recent results showed that the toxohormone content of a cancer tissue can be markedly increased by injections of certain amino acid mixtures into cancer bearing animals, and it was ascertained that eight specific amino acids are concerned in the process. A brief account of these experiments and discussion as to the possible significance of the results are presented in this paper.

METHODS FOR THE ESTIMATION OF RELATIVE TOXOHORMONE CONTENTS OF TUMOR TISSUES

Without making any attempt to isolate the active substance, Adams (5) in 1950, arrived at the same conclusion as ours regarding the existence of the toxic substance in cancer tissue which depresses the liver catalase activity. He showed that following the subcutaneous injection of homogenated mouse tumor, the liver catalase activity of normal mice markedly diminished at 24–48 hours, subsequently

rose to normal by the 4th day, and fell again during the growth of the new tumor arizing from the injected homogenate. Injections of variety of normal tissues produced no material change of liver catalase level. A significant fact was noted that with three mouse sarcomata (sarcoma 37, sarcoma 2146 and Crocker sarcoma 180) 50 mg homogenate was quite sufficient to produce a definite depression of liver catalase, carcinoma 63 at the same dose produced an appreciably smaller depression, while four rat tumors (sarcoma RIB 1, sarcoma RIB 5, Jensen sarcoma and experimentally produced primary hepatoma) and three human tumors (oat-cell carcinoma of lung, rectal carcinoma and breast carcinoma) had no effect in 100 mg doses. These observations, showing as they do that the amount of homogenate necessary for the production of a definite depression of liver catalase varys according to the tumors, suggested to us a simple method for the rough comparison of the toxohormone contents of tumor tissues.

Toxohormone Activity of NF Mouse Sarcoma Tissue as Determined by the Homogenate Injection Method

In all the experiments to be described in this paper, a transplantable mouse sarcoma, termed NF, was used. This strain was first discovered by us in 1948 and has been maintained through serial transplantations in hybrid mice now for over 90 generations. For the description of the tumor the reader is referred to the third paper of this series of studies (3). It has already been demonstrated that mice bearing NF sarcoma show the characteristic low liver catalase activity, and that potent toxohormone fractions can be isolated from the sarcoma tissue by the usual method of extraction with water, precipitation with alcohol and reprecipitation with normal HCl. It must be noted, however, that the sarcoma is apparently of low malignancy in that it permits mice bearing it to live in good physical conditions until the tumor grows to a huge size.

Freshly removed sarcoma tissue was trimed free of connective tissue stroma and any necrotic part, and was homogenized using a homogenizer of Elvejhem-Potter type with an appropriate amount of sterile saline solution under surgically aseptic conditions. The homogenate was then injected intraperitoneally in varying amounts into normal mice.

All the tumor bearing and normal mice here used were of a mixed albino strain, and were maintained on the usual laboratory diet of mixed grains with occasional supply of dried fish and green vegetables.

Twenty-four hours after the injection, the mice were killed by exsanguination, and the catalase activity of liver was determined gas-volumetrically, exactly as in all our previous experiments.

In Table 1 are given the results of this experiment. The doses of the tumor

homogenate are expressed in the term of the equivalents of the original tumor tissue, and the liver catalase activity represented by cc of oxygen produced under certain identical conditions, details of which need not here be repeated.

Table 1. Liver catalase activity of mice injected with varying amounts of sarcoma homogenate.

Amount of sarcoma homogenate injected. mg	Mouse No.	Liver catalase activity Oxygen cc
0	1	7.9
〃	2	7.8
〃	3	6.5
〃	4	6.1
〃	5	5.9
〃	6	5.8
〃	7	4.6
50	8	6.7
〃	9	5.4
100	10	8.6
〃	11	7.6
400	12	6.2
〃	13	6.2
〃	14	5.7
〃	15	5.6
500	16	5.2
〃	17	5.0
〃	18	4.3
〃	19	4.0
600	20	4.6
〃	21	3.2
700	22	4.2
800	23	3.5
〃	24	3.5
〃	25	2.9

The above results show that at least 600 to 800 mg of NF sarcoma tissue must be injected into normal mice in order to depress their liver catalase activity beyond the limit of the normal variability, which is in our term 4.0 cc oxygen. 800 mg produced a very clean-cut depression, while there was no depression after injections of 400 mg or less.

In view of the relatively small doses in which homogenate of certain other mouse tumors produced the liver catalase depression (Adams), it is obvious that the NF sarcoma is of low toxohormone content. This fact should be kept in mind,

since any experimental attempt to increase the toxohormone content may fail with tumors which "normally" have very high contents.

Effect of Protein Hydrolysates on the Toxohormone Content of Tumor Tissue

In the following experiments we found that the toxohormone content of the NF sarcoma tissue can be increased two to three folds by injecting into the mouse bearing it a solution of protein hydrolysate.

In the first experiment the material obtained by digesting codfish protein according to the usual method of hydrochloric acid hydrolysis was used. It was injected as distilled water solution into mice bearing 2~3 week old well established grafts of NF sarcoma in five daily doses of 50 mg each, totalling 250 mg. 48 hours after the last injection the mice were sacrificed, the sarcoma tissue homogenized in the same way as before, and homogenate was injected intraperitoneally into normal mice in varying amounts. The liver catalase activity of these mice was estimated 24 hours after the homogenate injection, according to our customary method.

As may be clear from Table 2, 200 to 400 mg tissue equivalents of sarcoma homogenate was found to be sufficient to markedly depress the liver catalase activity.

Table 2. Toxohormone potency of sarcoma tissue from mice injected with fish protein hydrolysate

Amount of sarcoma homogenate injected. mg	Mouse No.	Liver catalase activity Oxygen cc
50	1	7.9
150	2	5.0
〃	3	4.9
〃	4	4.2
200	5	3.7
〃	6	3.1
〃	7	2.9
400	8	3.0
〃	9	2.8
〃	10	2.6
〃	11	2.4

The above experiment was repeated, using on this second occasion hydrolysate of blood protein, prepared by hydrolyzing dried ox blood by means of baryta, all other experimental conditions being the same.

The result, as shown in Table 3, demonstrated that alkali digest of blood was as effective as acid hydrolysate of fish protein in increasing the toxohormone potency of sarcoma tissue.

Table 3. Toxohormone potency of sarcoma tissue from mice injected with alkali hydrolysate of blood protein

Amount of sarcoma homogenate injected. mg	Mouse No.	Liver catalase activity Oxygen cc
200	1	4.5
〃	2	3.8
〃	3	2.7
400	4	3.7
〃	5	2.8
〃	6	2.6
〃	7	1.8

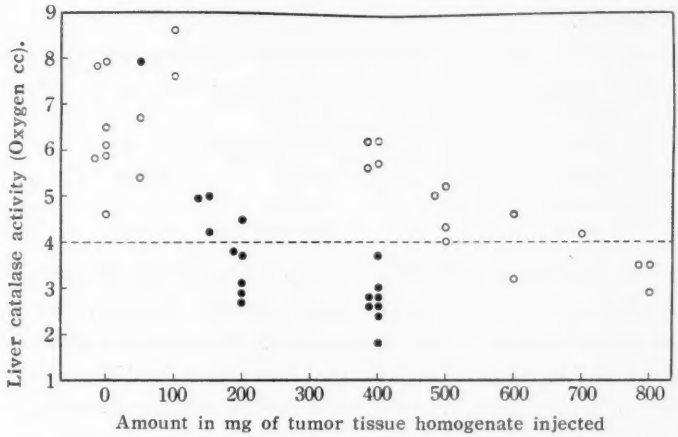

Chart 1. Liver catalase activity of mice injected with tumor tissue homogenate.
○——Tumor tissue from untreated mice.
●——Tumor tissue from mice injected with protein hydrolysates.

Effect of Amino Acid Mixtures on the Toxohormone Content of Tumor Tissue

The protein hydrolysates used in the above experiments are complex mixtures, but we ventured to assume, for the purpose of further experiments, that the substances responsible for the increase of the toxohormone activity of tumor tissue may be amino acids. This assumption is based on our present contention that toxohormone may be a kind of polypeptide, and on the corollary that a certain number of amino acids may constitute its building material.

Theoretically, the toxohormone-increasing effect demonstrated in the preceding experiments is capable of two alternative interpretations, namely, either that the injections of protein digest stimulated the toxohormone producing function of the

tumor cells, or that the digest supplied to the tumor cells extra quantity of material from which to synthesize toxohormone. We chose to approach the problem from the point of view of the second interpretation.

In selecting amino acids to be tested in the following experiments we were guided by the work of Greenfield and Meister (6) who reported the presence of the following amino acids in the digest of a crude toxohormone concentrate (alcohol precipitate) : alanine, glycine, serine, proline, aspartic acid, arginine, valine, threonine, phenylalanine, hydroxyproline, α–aminobutyric acid, isoleucine, tryptophan, lysine, leucine, and glutamic acid. Of these amino acids, tryptophan was excluded because of the activity of our acid hydrolysate. Hydroxyproline, α–aminobutyric acid and isoleucine were not available for us and had to be omitted. It was therefore with the remaining 12 amino acids that we started our next series of experiments.

The amount of each amino acid incorporated into mixtures was as follows:

Threonine	20 mg	Aspartic acid	20 mg
Serine	〃 〃	Arginine	〃 〃
Valine	〃 〃	Phenylalanine	〃 〃
Glycine	〃 〃	Lysine	40 mg
Alanine	〃 〃	Leucine	〃 〃
Proline	〃 〃	Glutamic acid	70 mg

All the samples used were crystalline l–amino acids, except threonine, serine, valine, phenylalanine, and lysine, which were synthetic dl-forms. For many of these samples we are indebted to the kindness of Mr. Sakae Emoto of the Scientific Research Institute and Mr. Chiharu Fukai of the Government Food Research Institute.

Each mixture of amino acids, varying from 230 to 330 mg dependiag on the mixture, was dissolved in 5 cc of distilled water by heating, neutralized to pH 7 with NaOH, and injected intraperitoneally into mice bearing NF sarcoma of a suitable size in 5 daily doses of 0.5 cc each.

The mice were killed 48 hours after the last injection, a certain weighed amount of tumor tissue was homogenized, and the homogenate was then injected intraperitoneally into normal mice in amounts equivalent to 200 or 400 mg of the original tumor tissue. These amounts were suggested as critical by previous experiments with digests. 24 hours after the homogenate injection, the mice were killed by exsanguination and the catalase activity of liver was determined under the identical experimental conditions as before.

A series of experiments were carried out as above described, varying the number of amino acids incorporated into the mixture, the amount of each amino acid being held constant. The results may be tabulated as follows:

Table 4. Effect on liver catalase activity of normal mice of tumor homogenate from mice treated with amino acid mixtures.

Amino acid mixtures	Amount of homogenate injected mg equivalent of tumor tissue	Mouse No.	Liver catalase activity Oxygen cc
I With the 12 amino acids	400	1	4.1
		2	3.3
		3	2.8
		4	2.6
II Same as I minus Threonine	400	5	4.0
		6	3.9
		7	3.3
		8	2.9
III Same as II minus serine	400	9	5.2
		10	4.2
		11	3.6
		12	3.2
IV Same as III minus valine	400	13	4.0
		14	3.6
		15	2.8
		16	2.7
V Same as IV minus glycine	200	17	5.9
		18	4.4
		19	4.3
		20	3.9
		21	3.5
		22	3.4
		23	3.0
		24	2.5
	400	25	4.8
		26	4.7
		27	4.2
		28	4.0
		29	3.6
		30	3.4
		31	3.3
		32	3.3
		33	2.8
		34	2.3
		35	2.1
		36	2.0
VI Same as V minus alanine	200	37	7.2
		38	7.0
		39	6.9

		40	6.7
		41	4.5
		42	4.4
	400	43	6.3
		44	5.5
		45	5.0
		46	4.6
		47	4.3
		48	3.5
VII Same as V minus proline	200	49	6.3
		50	6.2
		51	4.6
	400	52	6.2
		53	5.8
		54	4.3
VIII Same as V minus aspartic acid	200	55	7.6
		56	4.7
	400	57	7.0
		58	6.6
		59	4.1
IX Same as V minus arginine	200	60	6.6
		61	5.7
		62	5.3
		63	4.2
		64	3.9
		65	3.6
	400	66	9.4
		67	7.0
		68	6.7
		69	5.1
		70	4.9
		71	4.5
		72	4.0
		73	3.4
		74	3.2
		75	3.1
X Same as V minus phenylalanine	400	76	8.7
		77	7.4
		78	5.7
XI Same as V minus lysine	400	79	5.6
		80	5.2
		81	4.8
		82	4.0

XII Same as V minus leucine	200	83 84 85	6.2 5.9 5.3
	400	86 87 88	6.2 5.8 5.6
XIII Same as V minus glutamic acid	200	89 90 91	7.2 4.4 3.8
	400	92 93 94	6.2 5.8 5.5

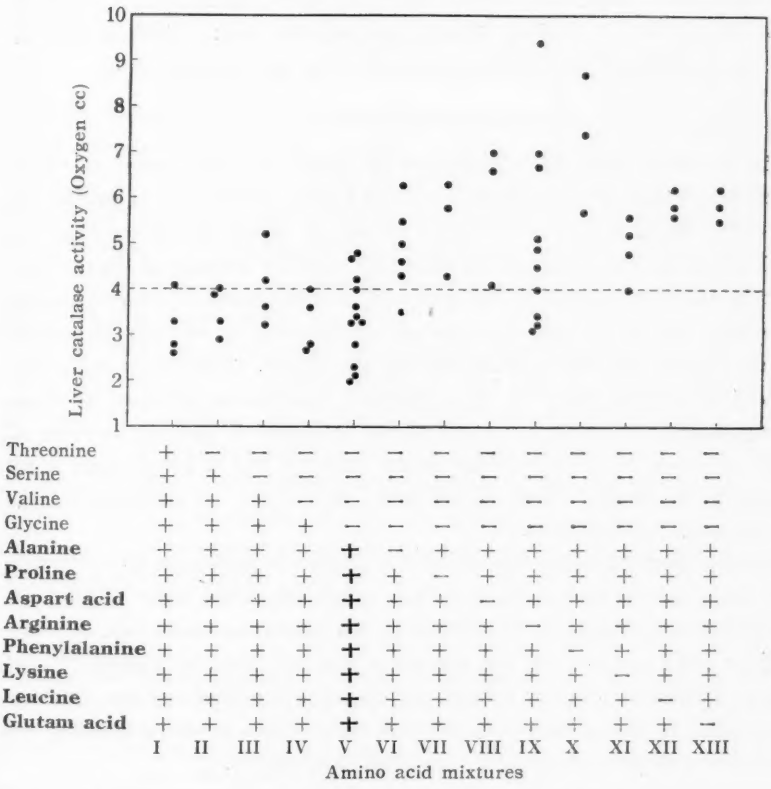

Chart 2. Liver catalase activity of mice injected with 400 mg tumor tissue homogenate from mice injected with various amino acid mixtures.

Of the 12 amino acids tested, threonine, serine, valine, and glycine do not seem necessary in order to increase the toxohormone content of the tumor tissue, since in the above experiments Mixture V, lacking these amino acids, was no less active than Mixtures I to IV containing any or all of these acids.

All the 8 amino acids composing Mixture V seem essential to the activity, and it may be noted that exclusion of any one of them more or less completely robbed the mixture of its toxohormone increasing potency (Mixtures VI–XIII). Arginine may possibly be an exception, as the effect of Mixture IX, lacking this amino acid, was not as clean-cut as in the cases of seven other mixtures. There is little doubt, however, that the potency of a mixture is distinctly higher with arginine than without it. Compare the effect of Mixtures V and IX in the above table.

It may be deduced from the above experiments that the toxohormone-increasing effect of protein hydrolysates upon tumor tissue can be reproduced by a mixture of crystalline amino acids, and further that only eight specific amino acids are required to bring about that effect. These amino acids are: Alanine, proline, aspartic acid, arginine, phenylalanine, lysine, leucine, and glutamic acid.

DISCUSSION

The fundamental concept which guided us in conducting the above described experiments has been that toxohormone may be a kind of polypeptide synthesized by cancer cells. Such a synthesis may be increased either by stimulating the synthetic function of cancer cells, or by supplying an extra quantity of the material for the synthesis. Our results clearly indicated that the toxohormone content of the tumor tissue can be markedly increased by injecting a mixture of certain specific amino acids into the animals bearing the tumor. The amino acids concerned are of no unusual type, all being normal constituents of most proteins. That the combination of these amino acids can stimulate a specific function of cancer cell (sythesis of toxohormone) seems unlikely, and it would seem much more reasonable to regard these amino acids as serving as material for the synthesis of specific polypeptide.

Even granting that the basic concept of the present experiments to be correct, we are far from certain that toxohormone is composed of the eight amino acids which we found to be essential for increasing the toxohormone content of tumor tissue. Many other amino acids are present in the free state in tumors (7) and all should be available to tumor cells if any of them is needed for the synthesis of toxohormone. It seems, however, that the eight amino acids in question are the ones that are required in greater quantities than others.

In this discussion it may be appropriate to refer to certain possibly important factors in our experiments which might have played unpremeditated roles. One of these is the fact that the NF mouse sarcoma, which we used exclusively,

happened to be very low in its toxohormone content, as much as 800 mg sarcoma tissue being required to produce unequivocal depression of liver catalase in normal mice. We have observed that mice bearing this sarcoma may live without outward signs of detelioration until the tumor grew to a size approaching that of the animal itself. There is a possibility that a tumor of low toxohormone content, such as this sarcoma, may respond more easily to external influences affecting the toxohormone activity, and not only the increase of toxohormone content by amino acid injections reported in this paper, but also the counteracting effect of excess iron which we previously noted (3), might have been difficult to demonstrate, had we been working with a tumor of maximum toxohormone potency.

Another point to be mentioned is the protein insufficient nature of the diet upon which our mice have been maintained throughout the experiments. It is possible that we have tested unintentionally the effect of amino acids under a relatively protein deficient condition, and if our diet contained adequate protein, the effect of the additional supply of certain specific amino acids might not have been easy to bring to light.

SUMMARY

Utilizing the method of injecting tissue homogenate for a rough estimation of relative toxohormone content, we first found that toxohormone content of tumor tissue can be markedly increased by injecting protein hydrolysates into animals bearing the tumor. It was then determined, by testing the effect of various amino acid mixtures, that eight specific amino acids were essential to this effect. These amino acids were: Alanine, proline, aspartic acid, arginine, phenylalanine, lysine, leucine, and glutamic acid.

The findings were interpreted as indicating that these amino acids may be the major components of toxohormone, and that the increase in the toxohormone content may be due to the supply of extra quantity of the material for the synthesis of this unique toxic product of tumor cells.

The "normally" low toxohormone content of the strain of tumor and the protein sub-deficient nature of the diet used in these experiments were pointed out as possibly important factors in revealing the role of the specific amino acids in toxohormone synthesis.

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要　旨

アミノ酸と癌組織によるトキソホルモンの合成

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われわれは癌の特殊毒性物質トキソホルモンは一種のポリペプチードであろうと推定しているが，今回担癌動物に蛋白水解物，あるいはアミノ酸混合物を注射すると，その癌組織のトキソホルモン含有量が著しく増加することを見出した。必要なアミノ酸はアラニン，プロリン，アスパラギン酸，アルギニン，フェニルアラニン，リヂン，ロイシン及びグルタミン酸の8種類で，トキソホルモン粗製標品の水解物中にはこの外になお多くのアミノ酸が検出されているが，他のアミノ酸は無関係である。

この所見から，これ等8種のアミノ酸が癌細胞によるトキソホルモンの合成に特殊な役割を演ずるものであることは疑ない。現在のところ，われわれはこれ等8種のアミノ酸がトキソホルモンの主要な構成物質であり，それを余分に供給することによつて癌細胞が多量のトキソホルモンを合成するものと解釈している。　（文部省科学研究費による）

[GANN, Vol. 44; March, 1953]

DIFFERENCE IN TISSUE AFFINITY BETWEEN O-AMINOAZOTOLUENE AND ITS "NON-CARCINOGENIC" ISOMER, P-AMINOAZOTOLUENE*

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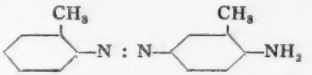
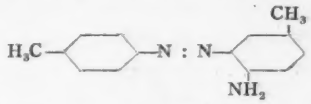
Cancer Institute (Japanese Foundation for Cancer Research), Tokyo

When Sasaki and Yoshida[1] first demonstrated the liver cancer producing effect of o-aminoazotoluene [I] they also referred to the fact that its position isomer, p-aminoazotoluene [II], fails to show the faintest indication of carcinogenic action.



[I]



[II]

This interesting observation led us to seek some difference in the biological action that may be found between the two isomers, since any difference discovered may conceivably serve as an enterring wedge into the question of the biochemical mechanism of cancer production. It was incidental to our efforts along this direction that we noticed a significant fact that feeding with p-aminoazotoluene brought about a beautiful vital staining of fat tissue, contrasted to no notable storage in this tissue of o-aminoazotoluene.

VITAL STAINING OF FAT TISSUE BY p-AMINOAZOTOLUENE (2)

The experimental method was simply to feed normal albino rats with the usual laboratory diet of unpolished rice, to which was added p-aminoazotoluene in vegetable oil at the rate of 0.6 percent. The rats killed and examined as early as 7 days after the beginning of the feeding showed the entire fat tissue throughout the body stained orange yellow, subcutaneous, mesenterial and all other fat tissues being equally well stained. Longer duration of feeding intensified the vital staining until the maximum seemed to be reached in 3 or 4 weeks. The staining appeared to be limited to fat tissue only; there was no staining of nervous tissue. Under similar experimental conditions, other fat staining dyes such as Sudan

* Experiments upon which the present paper is based were conducted during 1943—44 and published in preliminary notes in Japanese (2~4). To this date we are unaware of any other report bringing out the point disclosed by these experiments.

III, scarlet red, Nile blue sulfate and methyl orange, were tested, but they failed to show recognizable vital staining of fat tissue, with the exception of Sudan III, which gave only a faint coloring.

Color Reaction of p-Aminoazotoluene (3)

An opportunity to make quantitative estimation of o- and p-aminoazotoluene in the liver and fat tissue arose when, in desultory experiments, we noticed that p-aminoazotoluene in ether solution gives a beautiful violet color upon the addition of sulphuric or hydrochloric acid ; o-aminoazotoluene turns orange red under the same treatment.

The method is to take the ether solution of the substance in a small test tube and add 2 or 3 drops of conc. sulphuric acid (or hydrochloric acid), but in order to render clear the mixture 1 or 2 more drops may be required. Neither excess of acid nor the presence of fatty substance in the mixture interfere with the color reaction.

The intensity of the violet color is proportinal to the concentration of p-aminoazotoluene and is recognizable positively up to the dilution of 0.0001 percent. The color tends to fade in a few hours, the reaction mixure becoming completely colorless in 20 hours.

Estimation of o- and p-Aminoazotoluene in Liver and Fat Tissue of the Dye Fed Rats (4)

Normal albino rats were fed on unpolished rice powder with the addition of either o- or p-aminoazotoluene at the rate of 0.6 percent. After 4 weeks rats were killed by exsanguination and liver and as much as easily practicable of fat tissue were removed and were ground into paste. A weighed amount, 3 or 4 g, of each tissue paste was thoroughly extracted with about 50 times the volume of ether. For the estimation of p-aminoazotoluene the liver extract, which appeared pale yellow, was "boiled" down to 2–4 cc, and fat tissue extract, strongly colored salmon pink, to 5–10 cc. In the case of o-aminoazotoluene both liver and fat tissue extracts were concentrated to 5–10 cc. These concentrated extracts were tested with a few drops of conc. sulphuric acid, and the intensity of the color produced was matched against those of the standard solutions of the respective substance. From the approximate concentration of the substance in the extract determined in this manner a rough estimate of the amount of the substance in the original tissue was arrived at, and the figures were recalculated for 100 g of the fresh tissue. In the following table we give the results of duplicate experiments :

Table 1. Estimated amounts in gamma of the two substances per 100 g of fresh tissue.

	o-Aminoazotoluene		p-Aminoazotoluene	
	Fat tissue	Liver	Liver	Fat tissue
Exp. 1	578	1363	Less than 100	1623
Exp. 2	662	1070	Less than 100	4000

VITAMIN A REACTION

Liver extracts from p-aminoazotoluene fed rats concentrated to a small volume showed the blue color characteristic of vitamin A when treated with sulphuric acid. This color was confined to the ether (oil) layer and the sulphuric acid layer below it was light brown. That this represented a real negative reaction for p-aminoazotoluene, not a masked reaction, was proved by the fact that the same liver extract, to which was added a trace of p-aminoazotoluene, reacted with the typical violet color. It is to be noted that the color reaction for p-aminoazotoluene is positively recognizable up to the dilution of 0.0001 percent.

In the case of the liver extract from o-aminoazotoluene fed rats the vitamin A reaction did not appear. The absence of vitamin A from liver cancer produced by o-aminoazotoluene feeding was previously reported by Goerner and Goerner[5] and it seems that vitamin A disappears from the liver fairly early in the course of the feeding.

CONCLUSION AND COMMENTS

We may conclude that under the identical conditions of feeding, o-aminoazotoluene, which produces liver cancer, is demonstrable in a considerable amount in the liver, but its allegedly non-carcinogenic isomer, p-aminoazotoluene, is deposited largely in fat tissue, the liver retaining the substance only in an undeterminable minute amount. The outstanding difference in the biological behavior between the two isomers seems to lie in their tissue affinity.

The failure of p-aminoazotoluene feeding to produce liver cancer may well be due to the fact that this substance is not retained in the liver in a sufficient concentration for the purpose, which is quite a different thing from the substance having no carcinogenicity. Previous workers discussed the relationship between the chemical structure of azo-compounds and their carcinogenicity, based merely on production or non-production of liver cancer when fed to rats. It is now demonstrated that the tissue affinity is an important factor in determining positive or negative liver cancer production, and from this point of view the cases of the alleged non-carcinogenic compounds would call for reinvestigation before they can be definitely pronounced as having no carcinogenicity.

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要　旨

o-Aminoazotoluene とその“非癌原性異性体” p-Aminoazotoluene との組織親和性の差異

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佐々木・吉田は o-Aminoazotoluene 飼与による肝癌生成実験において，その位置異性体 p-Aminoazotoluene が同じ要約の下に“発癌性の片鱗をも示さない”ことを報告した。われわれはこれら両異性体の生物学的作用を比較検討することによつて，肝癌生成の生化学的機転に関し有意義な手懸りが得られるかと考えたのであるが，本文記述のように，o-Aminoazotoluene は相当大量肝臓に証明されるに反し，p-Aminoazotoluene は大部分が脂肪組織に沈著して美しい脂肪組織の生体染色を示すが，肝臓には確実に証明し得ないことが判明した。すなはち，p-Aminoazotoluene の“非癌原性”は，その十分な量が肝細胞に作用し得ないためであると説明することが出来る。物質に癌原性が有るかないかの問題ではなく，組織親和性の差異に由る食い違いである。

この研究は終戦前に行われ，結果は予報的に既に日本文で発表したものであるが，今日まで同様の所見が他所から報告されたことはないようである。

(文部省科学研究費による)

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FOURTH REPORT ON THE K. I. K. REACTION: FURTHER STUDIES ON THE PROPERTY OF K. I. K. FACTOR IN THE STOMACH JUICE FROM STOMACH CANCER PATIENTS

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It has long been presumed by old investigators that cancer tissue may produce some toxic substance which induces cachexia in the cancerous organism at the last stage of the disease. Since 1900 many studies were made by physicians but without a great success.

The fact that the so-called cancerous toxins reported by various investigators differ both in their effects and in their characters probably means that there might be various different toxic substances. Indeed, a series of such toxic substances were found in the cancer tissue, such as hemolysin (Kullmann, 1904, Grafe and Böhmer, 1908), splenomegalizing factor (Roffo, 1935, Sakai, 1939), blood pressure depressing factor (Ogura, 1925), miscarrying factor (Elsasser, 1939), toxohormone (Nakahara and Fukuoka, 1948) and finally K. I. K. factor (Kozawa, Iwatsuru and Kawaguchi, 1937).

These toxins not only exhibit such manifold effects, but their physical and chemical properties are different. Nakahara and Fukuoka's toxohormone is a thermostable, non heat-coagulable, water soluble, alcohol-precipitable protein-like substance. Kullmann's hemolytic toxin is thermolabil and soluble in both water and alcohol.

With regard to the anemiogenic factor, we have reported that it may be a protein-like substance, more accurately a polypeptide-like substance, as demonstrated in the experiments to be reported in this paper. In 1948, Sei claimed from his experiment that the anemiogenic factor from cancer tissue is an inorganic substance, i. e., $MgNH_4PO_46H_2O$. This salt, according to our study, indeed has anemiogenic effect, but we have the ground to believe that this effect is to be attributed to Mg ion. With this problem we will deal on another occasion, and in this paper the results of further studies on the properties of the K. I. K. factor made since our previous report will be described.

EXPERIMENTS

The methods for obtaining gastric juice, its neutralization, condensation, storage and the technique of the K. I. K. reaction were described in the previous papers, and so may be omitted here.

The following experiments demonstrate that the K. I. K. factor is not precipitable by trichloracetic acid.

To cancerous gastric juice, neutralized, filtered and condensed to about 20 cc, was added 1/3 volume of 20% trichloracetic acid, and centrifuged to separate supernatant and precipitate, and the latter dissolved in N/10 NaOH. Both solutions were dialyzed for 48 hours to remove trichloracetic acid, the removal of which being ascertained by adding white of egg without producing turbidity. The material thus obtained was again condensed to 5–10 cc, and its anemiogenic effect was tested on the rabbit.

Case No.	K. I. K. reaction		
	Methanol precipitate	Trichloracetic acid supernatant	Precipitate
1	−18%(+)	not tested	−2%(0)
2	−18%(+)	−22%(+)	0%(0)
3	−13%(+)	− 5%(0)	increased
4	−10%(+)	−13%(+)	increased
5	−14%(+)	not tested	increased
6	−14%(+)	− 4%(0)	increased
7	−14%(+)	A) − 6%(±) B) increased	increased
8	−13%(+)	−11%(+)	increased

The results are shown in the table above. In these tests the amounts of gastric juice used were as follows, case 1, 50 cc; case 2, 40 cc; case, 3, 24 cc; case 4, 30 cc; case 5, 25 cc; case 6, 20 cc; case 7, 20 cc. In cases 2 and 4, about half volume of the material was taken and the remainder used for paper chromatography. In case 7, while the supernatant was condensed and refrigerated, fine cylinder-like precipitate (A) was produced, which was centrifuged to separate supernatant (B). The most part of both (A) and (B) were used for paper partition chromatography. In case 8, extract of cancer tissue was used as a substitute for gastric juice.

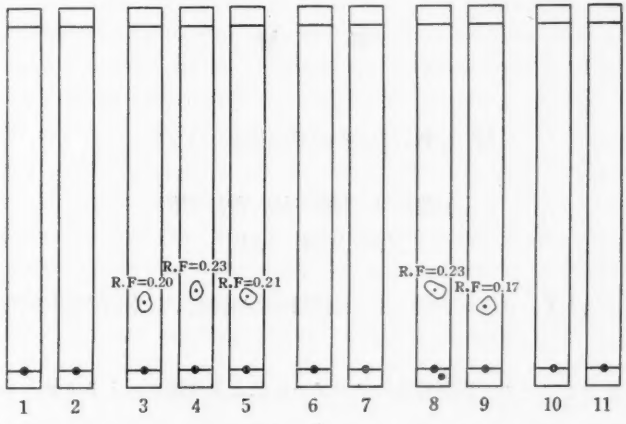
Judging from the above mentioned experiments, the anemiogenic activity was all positive in methanol precipitate, and all negative in trichloracetic acid precipitate, and as for trichloracetic acid supernatant, 3 were positive, 2 doubtfuly

positive, and 1 negative. The cause of negative results of these 3 cases are probably to be attributed to the relatively small amount of the available gastric juice, most part of the material being used for paper chromatography, so that the remaining material was too small in amount to reveal their anemiogenic effect. Anyhow these experiments show that K. I. K. factor is not precipitated by trichloracetic acid.

Paper partition chromatographic study of K. I. K. factor.

We employed one dimentional method, ascending form, using No. 2 filter paper manufactured by Toyo Roshi Co., and buthanol acidified with acetic acid as solvent.

The chromatograms are shown in the following figures, which prove that methanol precipitate and trichloracetic acid supernatant show a quite similar spot. In the figure, 1 is that of methanol precipitate from normal gastric juice, 2, methanol supernatant from normal gastric juice. 3, 4 and 5, methanol precipitate from cancerous gastric juice, 6, trichloracetic acid precipitate from normal gastric juice, 7, trichloracetic acid supernatant from normal gastric juice, 8 and 9, trichlor acetic acid supernatant from cancerous gastric juice, 10 and 11, trichloracetic acid precipitate from cancerous gastric juice.



CONCLUSIONS

K. I. K. factor is not precipitated by trichloracetic acid.

In paper chromatogram trichloracetic acid supernatant and methanol precipitate show a similar spot, which means that this spot corresponds to K. I. K. factor. Trichloracetic acid can precipitate ribo-nucleic acid, desoxy-nucleic acid and proteins higher than peptone, but peptide lower than peptone are not precipitated by this acid. These facts and the results of our former experiments on the pro-

perties of the K. I. K. factor made us believe that this factor is probably a polypeptide of lower grade.

Toxohormone (Nakahara and Fukuoka) is precipitated with methanol and also with trichloracetic acid, which is the character based on which the purification of this substance was performed. Both toxohormone and K. I. K. factor have some similarities, but at least in this point they are dissimilar, and we consider K. I. K. factor to be a peptide lower than toxohormone.

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要　旨

K. I. K. 因子の性状について

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K. I. K. 因子はトリクロール醋酸によつて沈澱せしめ得ない。本因子は低級なペプチッドと考えられる。

ペーパークロマトグラムで胃癌胃液のメタノールによる沈澱と，トリクロール醋酸を加えた際の上澄液とは同一のスポットを現わし，トリクロール醋酸による沈澱は何等のスポットをも生じない。

(文部省科学研究費による)

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STUDIES OF AMITOSIS WITH THE YOSHIDA SARCOMA*

(With Plates I–II)

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There are two ways, as generally accepted, of cell multiplication, namely, by mitosis and amitosis.

In mitosis it is evident that the cytoplasmic division follows the nuclear division, that is, one cell divides actually into two. In amitosis, however, the process is not so clear. First of all, it is very uncertain whether or not the cytoplasmic division actually follows the direct nuclear division or segmentation. Moreover, though the "amitotic figures" with furrows or constrictions of nuclei in stained preparations are usually interpreted as figures of the process of direct nuclear division, the interpretation is not supplied with firm basis of exact observations, since it is not sure toward which direction the furrows will progress, division or re-rounding, or whether it remains unchanged for a long time.

The concept of "amitosis" is very popular, but as to its real biological meaning there are many problems unsettled. Nuclear states or figures commonly regarded as "amitotic" in stained preparations, either in smear or section, require more discussion, especially from the view-point of cell multiplication, that is, whether they can actually contribute to the reproduction or proliferation of cells in normal or pathological tissue, including neoplasms.

The Yoshida sarcoma[1] supplies a very suitable material for the studies of amitosis or nuclear transformations. The tumor grows very rapidly and shows numerous mitotic and amitotic figures. I studied in the smear preparations of the tumor ascites abnormal nuclear shapes varied hand in hand with the tumor growth. At the same time the tumor ascites, i. e., a suspension of free tumor cells, is suitable for observations of tumor cells in their living condition under the phasemicroscope. I pursued in this way the variability of figures of resting nuclei and of those regarded to be in the course of amitosis.

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Experiments and Methods

(1) Observations of the smear preparations of the tumor ascites.

A droplet of the tumor ascites was obtained once a day after the transplantation of the tumor until the death of tumor animals and the smear of the ascites was stained with Giemsa solution. The numbers of mitosis, amitosis, and multinucleate cells among 5,000 tumor cells were calculated respectively and they were presented in several percentages. Various amitotic figures found in these preparations were described.

(2) Observations of amitosis in living condition.

Small droplets of tumor ascites obtained from the tumor rats near the last stage of the tumor growth were put on thin glasses and covered lightly with cover-glasses, all sides of which were sealed with melted paraffin. Nuclear shapes of tumor cells in the preparations and their variability in their living condition were examined under the phasemicroscope placed in the box kept at about 37°C.

Results and Discussion

(1) Morphology of amitosis

Though it is a difficult problem to decide what figures are characteristics of amitosis in fixed and stained preparation, the one which is narrowly furrowed from both sides at the middle part of the nucleus as shown in A of Diagram 1, may be regarded as a typical picture. It may be called dumb-bell shape or

Diagram 1. Amitotic figures

	(A) Dumb-bell shape	(B) Lobed shape	(C) One-side-furrowed shape	(D) Bud-sprouting shape
Nuclei that seem to divide into 2				
Nuclei that seem to divide into 3				

guitar shape (Figg. 1–5 in Plate I). Besides this dumb-bell shape .three other shapes with a narrow furrow are suggestive of amitosis. They are lobed shape (Diagr. 1. B and Figg. 6–10, Plate I), one-side-furrowed shape (Diagr. 1. C and Figg. 11–13, Plate I), and bud-sprouting shape (Diagr. 1. D and Figg. 14–15, Plate I). It is, then, a question, if there is any essential biological difference among these four groups of amitotic figures. Concerning this problem I want to point out first of all the following two points:

(a) In the case of dumb-bell shaped nuclei, we cannot help recognizing that they are in the course of complete separation (Figg. 3–5), but this is not the same case with the other three forms.

(b) Only in the case of dumb-bell shaped nuclei, the size of their cell-bodies is larger than that of ordinary mononucleate tumor cells (Tab. 1), while in the case of the other three shapes this is not so. (The size of tumor cells with abnormal nuclei shall be treated below in detail.)

The two points described above may supply a basis for accepting that the dumb-bell shaped nuclei have considerably different biological meanings from the other three abnormal nuclear shapes. In this connection it may be noted that the so-called pseudoamitosis though it may resemble in its shape to the dumb-bell shaped nucleus already discussed is originally an abortive "mitosis" and is different from amitosis in general.

(2) The size of cells containing abnormal shape nuclei

A measuring of the size of amitotic cells seems to be an important subject, though I am not yet informed that such an attempt has been done by any other researcher. I tried the measuring of them and compared the cell sizes of the four nuclear groups.

The diameters of the amitotic cells in the well stained smear preparations were measured, both in length and breadth of the cytoplasm by the micrometer, and the results were presented in the arithmetical average value for each of the four groups. On the other hand the diameters of several resting tumor cells near by them were measured similarly and then the mean value was also calculated. The results are shown in Table 1. The arithmetical average value of cytoplasmic diameters of 16 tumor cells having dumb-bell shaped nuclei dividing into two and that of 13 cells containing dumb-bell shaped nuclei dividing into three are 21.98 μ (13.33 to 33.75 μ) and 25.27 μ (16.06 to 31.77 μ) respectively. On the other hand the average value of cytoplasmic diameters of 76 ordinary (mononucleate) tumor cells is 17.24 μ (10.50 to 24.38 μ). From statistic investigations the former two have a significant difference from the latter. Therefore it may be safe to say that cells having dumb-bell shaped nuclei are larger in size than ordinary tumor cells. But this is not the same case with the other three nuclear shapes. The result implies that larger cells which accordingly have larger nuclei to a certain extent are apt to divide amitotically and present in the process dumb-bell shaped nuclei.

(3) Relation between amitosis and mitosis

After the inoculation of the tumor until the death of the animals the percentages of mitosis, amitosis, and multinucleate cells were calculated daily as described above.

(a) Daily change of percentage of mitosis:

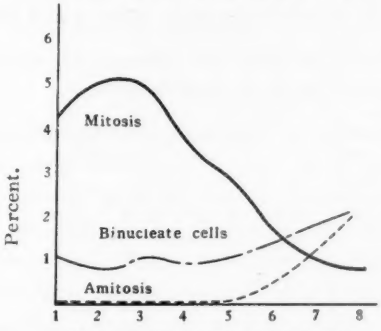
Table 1. Sizes of cells in amitosis and those of resting tumor cells of the Yoshida sarcoma

Resting tumor cells	Cells in amitosis				
	Nuclear shapes	Dumb-bell shape	Lobed shape	Bud-sprouting shape	One-side-furrowed shape
10.50, 17.21 μ		13.33 μ	13.26 μ	16.04 μ	18.75 μ
11.54, 17.29		16.06	18.33	16.88	19.46
12.63, 17.33		18.08	19.17	18.70	20.08
12.92, 17.38	Nuclei that seem to divide into 2	18.96	20.21	19.04	24.96
13.33, 17.46		19.17	24.80	20.00	26.04
13.38, 17.50		19.44		20.00	
13.42, 17.66		19.58		21.25	
13.54, 17.83		19.77		21.25	
13.63, 17.88		20.79		21.76	
13.75, 17.92		22.42		22.08	
13.83, 18.25		23.63			
13.88, 18.30		24.17			
14.17, 18.33		26.38			
14.58, 18.63		26.46			
14.63, 18.66		29.74			
14.80, 18.75		33.75			
14.88, 18.96		Average value			
15.00, 19.13					
15.21, 19.17					
15.33, 19.25		21.98	19.15	19.70	21.86
15.41, 19.38		Reliant limit in 95%			
15.42, 20.00					
15.63, 20.08		19.21～24.76	14.02～24.29	18.63～20.77	17.68～26.04
15.67, 20.25					
15.75, 20.41	Nuclei that seem to divide into 3	16.06	13.60		13.75
15.92, 20.42		19.58	13.75		15.00
16.04, 20.83		21.67	15.81		16.67
16.13, 21.25		22.58	15.98		16.88
16.25, 21.36		23.49	16.42		18.33
16.58, 21.46		23.99	17.68		20.06
16.66, 21.58		26.80	17.85		21.00
16.67, 21.75		27.29	20.63		23.29
16.83, 22.08		28.04	23.97		23.38
16.88, 22.63		28.96	25.00		23.75
16.96, 23.08		30.42	28.05		
17.08, 23.38		31.77			
17.16, 23.88					
17.17, 24.38		Average value			
Average value					
17.37		25.27	18.98		19.21
95% reliant limit		Reliant limit in 95%			
16.68～18.07		22.53～27.99	15.05～22.90		16.95～21.49

Table 2. Daily change of percentage of the mitosis in the tumor ascites of the Yoshida sarcoma

Animal No.		Days after the transplantation							
		1	2	3	4	5	6	7	8
									%
No. 1	Prophase	0.66	1.28	1.34	0.84	0.40	0.24	0.22	0.18
	Metaphase	0.76	1.42	1.14	0.86	0.82	0.38	0.42	0.13
	Anaphase	0.22	0.20	0.18	0.10	0.08	0.12	0.00	0.00
	Telophase	1.50	1.86	3.04	1.28	1.20	0.46	0.24	0.00
	Total	3.14	4.76	5.70	3.08	2.50	1.20	0.88	0.31
No. 2	Prophase	1.20	1.18	1.40	1.14	0.84	0.62	0.57	0.25
	Metaphase	1.50	1.24	1.56	1.32	1.00	0.76	0.50	0.30
	Anaphase	0.83	0.52	0.36	0.46	0.28	0.10	0.13	0.05
	Telophase	1.96	1.56	2.06	1.60	1.72	0.80	0.33	0.45
	Total	4.49	4.50	5.38	4.52	3.84	2.28	1.53	1.05
No. 3	Prophase	1.50	1.70	1.06	0.74	0.52	0.74	0.30	0.20
	Metaphase	1.50	1.48	1.10	0.88	0.78	0.28	0.10	0.23
	Anaphase	0.20	0.46	0.16	0.22	0.16	0.06	0.06	0.75
	Telophase	1.60	1.76	1.66	1.36	1.18	0.94	0.84	0.37
	Total	4.90	5.40	3.98	3.20	2.64	2.02	1.30	0.87
Average percentage of the mitosis in 3 cases		4.17	4.88	5.02	3.60	2.99	1.83	1.24	0.74

Diagram 2. Curves of daily changes of percentage of mitosis, and binucleate cells in the Yoshida sarcoma (These curves were drawn from the data indicated in Tables 2, 3, and 4.)



Mitotic figures, as shown in Tab. 2 and Diagr. 2, are very plentiful in number

(4 to 6%) in the early stage (1st to 4th day after the transplantation). In most cases they arrive at the maximum on the third or fourth day. In the middle stage (5th to 7th day), however, they are decreased indicating 2 to 3%, and in the most advanced stage (8th to 10th day) they decrease further to about 1% or so.

(b) Daily change of percentage of amitosis:

As indicated in Tab. 3 and Diagr. 2, amitotic figures which seem to divide into

Table 3. Daily change of percentage of the amitosis in the tumor ascites of the Yoshida sarcoma

Sort of shapes	Animal No.	Days after the transplantation							
		1	2	3	4	5	6	7	8
Nucleus that seems to divide into 2	No. 1	0.22	0.20	0.02	0.06	0.02	0.78	2.56	3.05 %
	No. 2	0.20	0.12	0.02	0.22	0.24	0.66	1.70	0.25
	No. 3	0.00	0.06	0.10	0.16	0.10	0.54	0.58	1.95
	Average	0.14	0.13	0.05	0.15	0.12	0.66	1.61	1.75
Nucleus that seems to divide into 3	No. 1	0.00	0.00	0.00	0.00	0.02	0.36	0.96	1.17
	No. 2	0.00	0.00	0.02	0.00	0.06	0.26	1.07	0.10
	No. 3	0.00	0.02	0.00	0.00	0.00	0.00	0.28	0.75
	Average	0.00	0.00	0.00	0.00	0.03	0.21	0.77	0.67

two or three parts are very few in the early and middle stages, while in the last stage they increase remarkably in number.

(c) Daily change of percentage of binucleate cells:

Generally speaking, the more nuclei a cell has, the lower the rate of its appearance becomes. Consequently the binucleate cells are those which we find most often among all polynucleate cells. The rate of their appearance is comparatively high (1 to 2%) in the early stage, decreasing a little in the middle staga, and then it increase again (2 to 3.5%) in the last stage (Tab. 4 and Diagr. 2).

Table 4. Daily change of percentage of binucleate cells in the tumor ascites of the Yoshida sarcoma

Animal No.	Days after the transplantation							
	1	2	3	4	5	6	7	8
No. 1	1.00	1.08	1.34	0.78	0.58	1.08	2.20	2.25 %
No. 2	1.66	0.50	0.74	1.40	1.90	2.44	2.66	3.15
No. 3	0.50	0.46	0.76	0.36	0.24	0.18	0.24	0.85
Average	1.05	0.68	0.94	0.85	0.94	1.23	1.70	2.08

Cells having more than three nuclei are very few, although they have a similar

tendency to binucleate cells to increase in the last stage.

Diagram 2 indicates that the amitosis and multinucleate cells surpass the mitosis in number toward the end stage of tumor development when the population of cells is dense and their living condition becomes worse.

(4) Relation between amitosis and multinucleate cells

As indicated in Diagr. 2 both binucleate cells and amitosis increase evidently in number in the progressed stage of tumor development. This seems to suggest that there is an intimate relation between the genesis of binucleate cells and amitosis. However, it is a problem if this is really so. I have already reported with Yoshida[2] and Sato the results of our phasemicroscopic observations that binucleate cells are often produced either by interruption of cytosomic division or by re-fusion of once separated daughter cells. I tried, therefore, further to observe the process of eventual formation of binucleate cells through amitosis in living condition under phasemicroscope.

Catching under the microscope various cells having deep furrowed or constricted nuclei as well as intact ones, I continued to observe each of them for two or three hours. Series of photographs of their nuclear and cytosomic changes during the observations were made.

Results are as follows:

1: Direct nuclear division:

In one of the ten cases I could confirm the direct nuclear division. In this case furrows were marked first at two different places of the nucleus. Each furrow became gradually deeper and narrower from both sides of the nucleus showing dumb-bell shape, and finally after about twenty minutes the nucleus divided perfectly into three pieces (Figg. 1–5, Plate II). Therefore it is evident that amitotic or direct nuclear division does exist, though it seems to be a very rare case.

2: Long staying constrictions:

In eight of the ten cases (two dumb-bell shaped, four lobed, and two one-side-furrowed nuclei), narrowly furrowed nuclei stayed in the almost same condition for a long time (1 to 2 hours), proceeding neither to separation nor to re-rounding of the constricted nucleus was noted. Though they may have ceased their activity on account of artificial condition in vitro, anyhow, such phenomenon was observed in the majority of my observations.

3: Recovery from nuclear constrictions:

It was observed in one case that a constricted nucleus resumed gradually a round shape. At first the furrows were being deepened, but deepening stopped and then after about an hour the nucleus became round again. Therefore it is demonstrated that nuclear constriction has a possibility of recovery (Figg. 6–9, Plate II).

4: A cell-body division following the direct nuclear division does not occur:

In smear preparations I could not find any sign of cytoplasmic division accompanying a direct nuclear division. This was further ascertained by the phasemicroscopic observations of cell-bodies in living condition.

It may be safe, therefore, to say that amitosis has no connection with cell multiplication, though it has a little bearing on the formation of polynucleate cells.

(5) Significance of amitosis

Does a cell really multiply by amitosis? This problem has been disputed by many authors with various materials for a long time, especially from the latter half of the nineteenth century toward the beginning of the twentieth century. In the literature there are various opinions on this problem. About two-thirds of writers, so far as I have gone over, believe the possibility of the proliferation of cells by amitosis, while the others regard it as a division limited only to nucleus, without cell multiplication. The opinions in the literature may be arranged as follows:

1: Amitosis is a sort of generative process accompanying cytosomic division (Strasburger,[3] Arnold,[4] Ströbe,[5] Meves,[6] Maximow,[7] Borst,[8] Kawanago,[9] etc.).

2: Amitosis has a cell-body division, but is not a generative process (Payne[10]).

3: Amitosis represents either a degeneration or an aberration and is on the road to ruin (Ziegler,[11] Flemming,[12] Krompecher,[13] Bast,[14] etc.).

4: Amitosis is performed for the purpose of expanding nuclear surface and is no sign of degeneration (Nakahara,[15] etc.).

5: Amitosis is an incomplete and abortive division (Klebs[16]).

6: Amitosis is a phenomenon produced by some special environmental conditions, for instance, when influenced by chemicals (Nathanson,[17] etc.).

Concerning my own studies, I could demonstrate, as above described, a cytoplasmic division that followed the amitosis or direct nuclear division neither in the smear preparations nor in the phasemicroscopic observations of cells in their living condition. Therefore, amitosis cannot be regarded as a process that contributes to cell multiplication. If the so-called amitosis, or amitotic nuclear changes as I prefer to say, do not actually contribute to the cell multiplication, what is the real biological meaning of such nuclear changes? My present studies did not make the meaning clear, but the changes do not seem to be only destined immediately to ruin. Their positive and progressive side may not be denied, but they are rather a regressive process, for they are inclined to increase toward the last stage of tumor development.

Summary

The process of the "amitosis" was studied with Yoshida sarcoma cells. Shapes of amitotic nuclei demonstrated in the smear preparations were classified into four

groups: dumb-bell shape, lobed shape, one-side-furrowed shape, and bud-sprouting shape. Among these, only in dumb-bell shaped nuclei it was confirmed that they could completely divide and present two or three separate nuclei. This was certified further by phasemicroscopic observations in the living condition.

Special attention was paid for the examination of cytoplasmic division following after the amitotic or direct nuclear division. However, it was demonstrated that any cytoplasmic division followed after a direct nuclear division neither in the smear preparations nor in the living condition of cells under the phasemicroscope. Therefore, it is a conclusion of present studies that the so-called amitosis does never contribute to cell multiplication, though it can bring about, only in rare cases, polynucleate cells.

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Explanation of Plate I

Amitosis and other abnormal nuclear figures in the smear preparations of the Yoshida sarcoma

Fig. 1: Nucleus narrowly furrowed from both sides (dumb-bell shaped nucleus) found in the 8th day tumor ascites after the transplantation.
Fig. 2: Ditto.
Fig. 3: Nucleus on the verge of division found in the 3rd day tumor ascites.
Fig. 4: Nucleus seems to divide into two found in the 8th day tumor ascites.
Fig. 5: Nucleus on the verge of division found in the 3rd day tumor ascites.
Fig. 6: Lobed shaped nucleus found in the 7th day tumor ascites.
Fig. 7: Ditto.
Fig. 8: Nucleus seems about to divide into three, 5th day tumor ascites.
Fig. 9: Nucleus with four lobes, 8th day tumor ascites.
Fig. 10: Nucleus with seven lobes, 8th day tumor ascites.
Fig. 11: One-side-furrowed nucleus, 2nd day tumor ascites.
Fig. 12: Nucleus deeply furrowed only from the outside, 8th day tumor ascites.
Fig. 13: Ditto.

Fig. 14: Bud-sprouting nucleus, 8th day tumor ascites.
Fig. 15: Ditto.

EXPLANATION OF PLATE II

Phasemicroscopic observations of direct nuclear division and nuclear transformation of Yoshida sarcoma cells in their living condition

(a) A kindney-shaped nucleus divided completely into three parts.
Fig. 1: 19°55′ A kidney-shaped nucleus.
Fig. 2: 19°57′ Furrows appeared.
Fig. 3: 20°00′ On the verge of division.
Fig. 4: 20°20′ A trinucleate cell presented.
Total lapse of time: 25 minutes

(b) A recurrence of irregular constriction of a nucleus.
Fig. 5: 18°10′ A somewhat irregularly furrowed nucleus.
Fig. 6: 18°26′ Furrows grew a little deeper.
Fig. 7: 18°40′ They further deepened.
Fig. 8: 19°40′ After one hour the nucleus was found perfectly round.
Total lapse of time: 90 minutes

要　旨

吉田肉腫による無糸分裂の研究

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高等動物細胞は主として有糸分裂によつて殖えるが，無糸分裂によつても殖えると言われる。原虫においては（あるいは細菌においても）無糸分裂による細胞体の増加は事実認められるが，高等動物の細胞の場合にも果して事実であるか否かの問題を追求して，熱海は吉田肉腫を用い，無糸分裂の研究をば次の二つの方法によつて行つた。すなわち第一は腫瘍移植後動物が死亡するまでの毎日の腫瘍腹水塗抹染色標本の逐時的観察である。第二は位相差顕微鏡を用いて腫瘍増殖末期腹水中の腫瘍細胞殊に核の生態観察である。塗抹標本観察によつて得た所見は。

(1)　無糸分裂では核が分れる事があつても細胞体は分れない。

(2)　異常核形を亜鈴形，分葉形，突起形，一方側縊れの四種に分類を試みると，亜鈴形においては核が無糸分裂的に分断すると確認されるが，他の形のものから核が分断して多核となる事は確認できない。

又異常形核を有する細胞の胞体直径を計測して見ると，亜鈴形核を有する細胞のみが一個の核を有する静止形腫瘍細胞よりも有意義の差を以つて大きい。

(3)　腫瘍増殖の初期には有糸分裂像が多くて無糸分裂像（上記の四つの核形）が少く，末期になると逆に有糸分裂像が減つて無糸分裂像が殖えてくる。

(4)　末期に異常の核形を有する細胞が多くなつても，それ程多核細胞は殖えない。

次に生態観察によつて得た所見は，

(1)　静止核の両側が縊れてすなわち亜鈴形を呈して核が二分する事実が確認された。（一例）

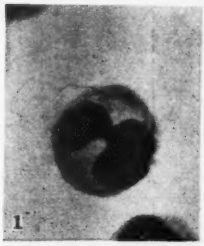
(2)　亜鈴形以外の異常の核形では縊れた状態を長く続けていて容易に分れない。（分葉形：四例。一方側縊れ：二例。）亜鈴形でも縊れが長く続いていて観察中には二分しなかつたものがある。（二例）

(3)　核の縊れは必ずしも分れる方向にのめ進行するのではない。逆に縊れが浅くなつたり，逆戻りする事もある。（一例）

(4)　無糸分裂で核は殖えるが，細胞の数は殖えない事は生態観察でも確認された。

以上の観察から無糸分裂を綜合的に考察して見ると，無糸分裂による細胞数の増加というものは存在しないと結論される。無糸分裂なる現象は細胞体の分裂を伴はない核のみの分裂及び

核の変形の一種である。亜鈴形核を有する細胞体は一個の静止核を有する細胞体より大きいが，分葉形・突起形・一方側縊れの核の場合には大きいとは言えない。且つ亜鈴形の場合は核が分れ得るのであるが，他の三者の場合は完全に分れる事実が確認出来ない。すなわち亜鈴形核は後三者とは多分に異る点があり，直接分裂型として認める事が出来るが，後三者は核の分裂型とは認め難く，生活条件による核の変形と考えるべきであろう。要するに所謂無糸分裂像には進行性あるいは積極的の意味は認め難く，退行性の現象とみるべきである。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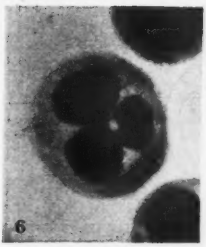
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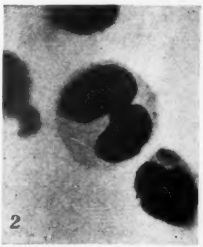
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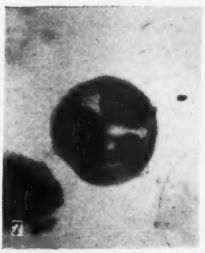
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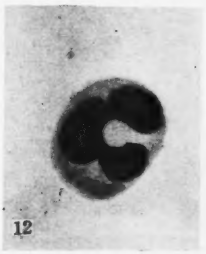
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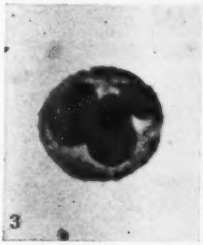
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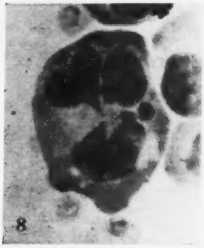
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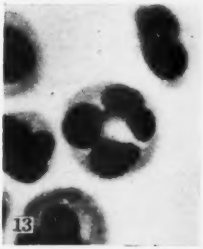
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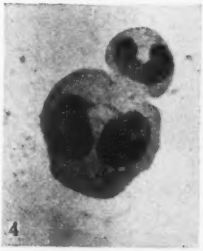
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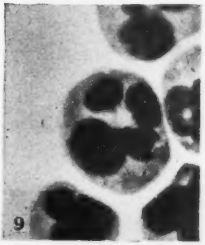
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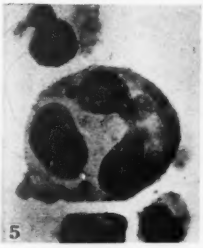
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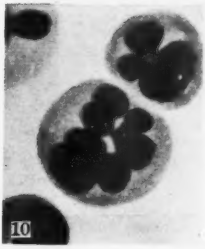
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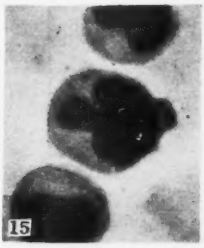
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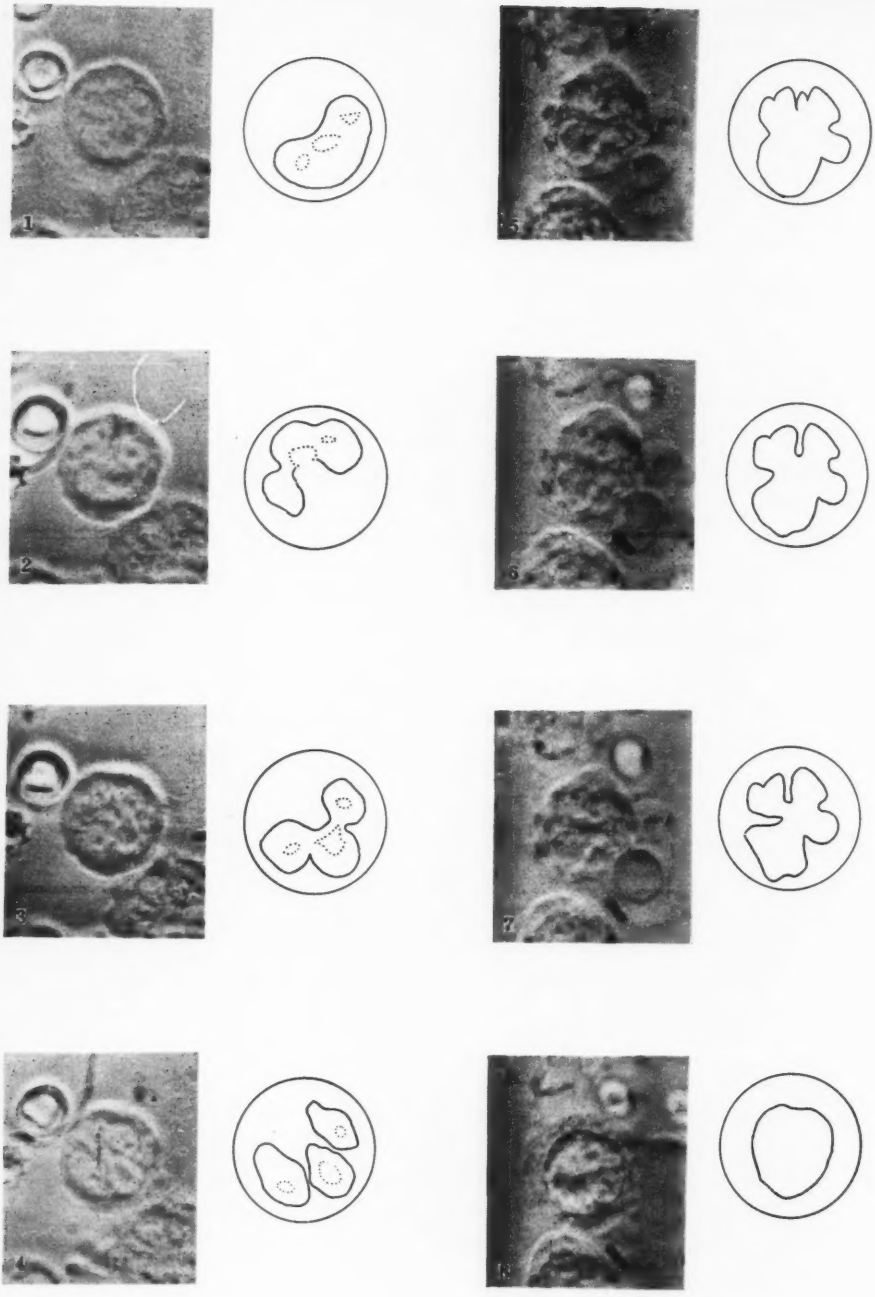

[GANN, Vol. 44; March, 1953]

ON THE HISTOGENESIS OF ADENOCARCINOMA OF THE STOMACH

(With Plates III and IV)

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In the already published paper,[1] one of the authors (T.M.) has reported and discussed on the histogenesis of a small solid cancer focus ($180\times150\times450\mu$) taken from an excised stomach specimen. Ninety serial sections were enough to examine the entire picture of this cancer focus. After further studies, a second solitary cancer focus was found. The authors wish to report and briefly discuss on the histogenesis of adenocarcinoma as this second lesion, which was found on the edge of a chronic ulcer, showed histological features of adenocarcinoma.

CASE REPORT

D.H., a 54 yr.-old man, case No. 263. Both clinical and roentgenological diagnosis being stomach ulcer, the stomach was excised on the 27 th of April, 1950 at the Fukuda Surgery, Tokyo University. Opening the excised specimen from the greater curvature, a 4.0×2.5 cm., oval shaped chronic ulcer (Fig. 1) was found on the lesser curvature 7 cm. distant from the pylorus. Etat mammelonne was marked in the surroundings. A small sphere found on the edge of the anterior wall of the ulcer was smooth, pink in color and villous. Specimens, which consisted of 29 blocks in all, were then taken from the center of the ulcer radially towards the edge. Carcinomatous changes were found histologically at the edge of the ulcer in 8 of the blocks. This coincided with the before mentioned pinkish villous lesion. As the sections of blocks A and B, adjacent to each other, showed the smallest cancerous lesions, serial sections (5μ thick) of all of block A was made in order to investigate the connection between blocks A and B (Fig. 2). Through these serial sections it was found that absolutely no connection existed between the cancerous lesions of A and B; the cancerous lesion of A gradually decreased in size and finally diminished to the minimum in the 70 th section and no further trace of cancer was met with in the following 330 sections (1650μ).

Serial sections of block B were then examined. The minute cancerous lesion gradually increased in size reaching its maximum area of $540\times380\mu$ between the

80 th and 100 th section. It then decreased in size and finally diminished to the minimum in the 190 th section and no signs of cancer were noticed in the following 200 sections. In other words, it can be said that this cancer focus is absolutely independant from the chief cancerous lesion and its width along the edge of the ulcer covered more than 190 sections or is about 1000μ (540×380×1000μ).

Figures 3 and 4 show section number 80, which is near the maximum cut surface area. The cancer focus exists near the point where the edge droops down toward and later into the base of the ulcer. They consist of many irregular glands and are connected in a mesh-like fashion. These glands are lined by a single layer of large basophilic cylidrical cells with large nuclei, prominant nucleoli and many mitotic figures. Marked infiltrations of wandering cells are found in and around the cancer focus; especially plasma cells prevail around the cancer focus and infiltration of polymorphonuclears are found within the lumina of the glands. Proliferation of connective tissue and development of capillaries are seen at the circumference of the focus. Cancer cells are already found at the outmost surface of the focus (Fig. 4), and their maximum width is shown also in sections 80-100.

By applying silver staining on section No. 106 (Fig. 5), it was found that the basement membrane was firm as a rule, but disorganized or lacking in some parts. From the above mentioned findings and that the chief cancer focus (block A in Fig. 2) consists of nearly identical glands, the glandular group can be pointed out as a cancer focus.

The characteristic feature found in section Nos. 80 and 106 is that numerous cancerous glands are convergent to one point of the gastric foveola (C in Fig. 4, and D in Fig. 5). Such convergent points are also found in section Nos. 14, 33, 56, 70, 86, 100, 130, 154 and 178. Most of these convergent points form points, but those found in Nos. 86, 100 and 130 are not points but rather a surface. In some sections (No. 70) two convergent points were found. There are some places in which marked cancerous changes have taken place at the epithelium of the convergent point and some with the entire surface lined by non-cancerous foveolar epithelium. In some places one convergent point was inseparable from an adjacent convergent point, and as in sections 60 and 166, there were no connections between the deeply implanted cancer focus and its covering epithelium.

Discussion

The authors now wish to discuss some points concerning the development of this cancer focus. As there exists a larger cancerous lesion in this case, it is quite a difficult task to fully prove that infiltration, metastasis or intracanalicular dissemination had not occurred. The interpretation that at the beginning a shallow chief cancer focus spread out with a part within falling off and leaving

a solitary cancerous islet can be denied as the surface epithelium of foveolar epithelium character as a whole is firm. There are also no proof of lymphatic invasion around the cancer focus. Haematogenic metastasis can also be denied as the patient is still living in good health two years after operation. There is no proof to deny the intracanalicular dissemination, but there remains a big question as to whether such a mode of cancer invasion exists.

From the above mentioned facts this small focus, even though a large cancer focus did exist nearby, can be called an adenocarcinoma in situ.

In general the developmental mechanism of adenocarcinoma is considered to be such[2] as that shown in the next diagram; the already existing glands give

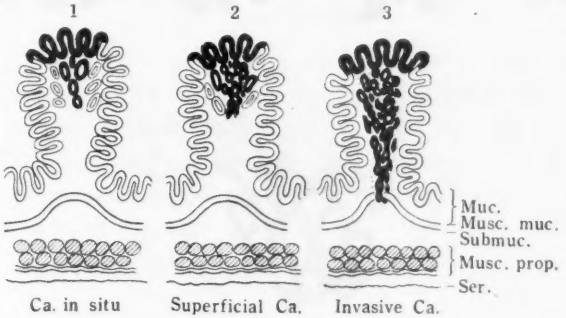

Diagram 1. A diagrammatic representation of polypus cancer[2]. 1 is cancer in situ which becomes a superficial cancer (2) when it begins to infiltrate into the lamina propria. 3 indicates an invasive cancer with the infiltration penetrating the muscularis mucosae.

rise to cancer in situ. Some parts of the basement membrane of the gland are then destroyed and at these points infiltrations into the lamina propria mucosae take place (superficial cancer). When this invasion extends into the submucosa after penetrating the muscularis mucosae it becomes an invasive cancer.

From the above mentioned point of view, the cancer focus in this case has already reached the stage of superficial cancer. Even after closely examining each section, there is no absolute proof as to which gland was first to give rise to cancer in situ or as to which basement membrane was primarily destroyed and invasion occurred from that point into the lamina propria mucosae. The only place which can be considered as a starting point for invasion with a break in the basement membrane is the before mentioned convergent point of the overlying epithelium of the surface. In other words, many cancerous glands have already spread radially into the lamina propria from this point.

Considering this problem from another point of view, the question arises as to whether cancer in situ really exists or not. It would be easy to understand the development of the cancer focus in this case presuming that a cancer in situ does

not exist or even if it does exist it is non-glandular in form with cancerous changes rising within a narrow extent of the covering epithelium coinciding with the convergent point, which can be considered as the developing point of the cancerous glands (diagram 2,1—8). A small portion of the covering

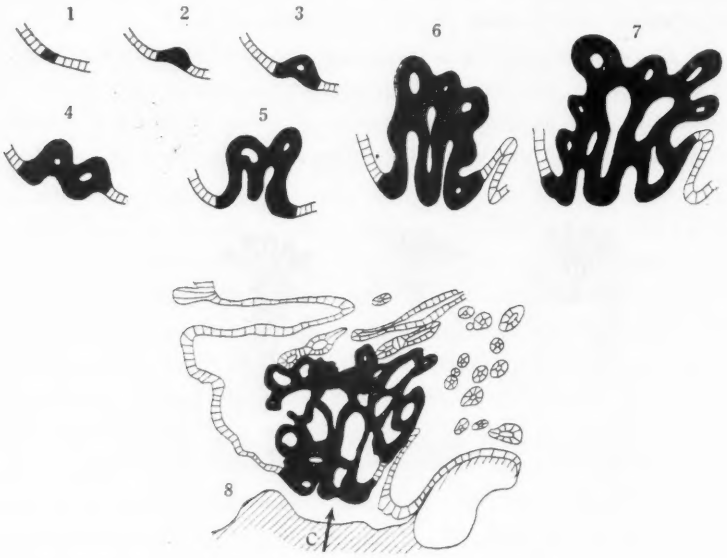

Diagram 2. Developing diagram (1-8) of serial section No. 80 (Fig. 4)
C indicates convergent point.

epithelium made up of foveolar epithelium[3] first shows cancerous changes and develops a cancerous gland into the lamina propria mucosae. In other words, adenocarcinoma at the beginning of its growth shows both atypy and infiltration at about the same period. This coincides with the developmental mechanism shown in solid cancer focus of the stomach already reported in another paper.[1]

In solid cancer many developing points in a single cancer focus were pointed out. The same thing can also be said in this case, except that there is still room for further consideration in deciding that the convergent points are all points for development. In adenocarcinoma the epithelium of developing points already showed cancerous changes and in some sections (Nos. 86, 100, 130) it has come to show a certain width in its spreading. Here we are already able to recognize a mechanism of the spreading of cancer; the replacement of non-cancerous epithelium by cancer cells which is seen in progressed adenocarcinoma. In this way the spreading cancer cells which replaced the covering epithelium may be considered also to develop glandular structures in the lamina propria mucosae.

Thus, some of the above mentioned convergent points may not perhaps be considered, stricktly speaking, as polycentric points of development. Especially, one must not forget this criticism when observing the portion where the deviding of one convergent point from the next one is incomplete even with serial sections. Thus, when speaking of polycentric points of development in this case, we must rely to some extent on its similarity to the polycentric nature of the development of solid cancer, which was more clearly analysed.

The authors wish to discuss the problems[4] (the depth of developing point, matrix tissue, etc.) on the histogenesis both in adenocarcinoma and solid cancer in another paper.

Conclusion

The entire picture of a small solitary adenocarcinoma ($380\times540\times1000\mu$) found in an excised stomach with a cancer focus in a portion of the edge of a large chronic ulcer was successfully clarified by careful observation of serial sections. Studies were made on the histological development of adenocarcinoma as this focus was considered as a cancer in situ, developed from the epithelium of the gastric foveola covering the edge of the ulcer.

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Explanation of Plates III and IV

Fig. 1. D.H., 54 yr.-old man, excised stomach opened from the greater curvature.

Fig. 2. In the drawn picture of the ulcer found in the center, only the black portion showed cancerous changes. Blocks A and B are located next to each other. The numbers here are the serial numbers of block B.

Fig. 3. Cut surface of serial section No. 90 of block B. Arrow indicates cancer focus. 8×4 power. H.E. stain.

Fig. 4. Higher power photomicrograph of Fig. 3. 8×10 power. H.E. stain. The cancerous glands are connected in a net-work with no solitary glands. C indicates convergent point.

Fig. 5. Silver stain of serial section No. 106. 8×10 power. D indicates convergent point. The basement membrane is mostly intact with but a portion lacking.

要　旨

胃の腺癌の組織発生について

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大きな慢性潰瘍の辺縁の一部が癌化している切除胃において，その癌化部を連続切片によつて詳細に調べた結果 380×540×1000μ の独立した小腺癌の全貌を明らかにすることが出来た。この癌は潰瘍縁を被う胃小窩小皮から，その場で発生した腺癌であるとみなすことが出来たので，先に報告した独立した充実癌の発生像と対比しつつ，腺癌の組織発生について，簡単な考察を試みた。

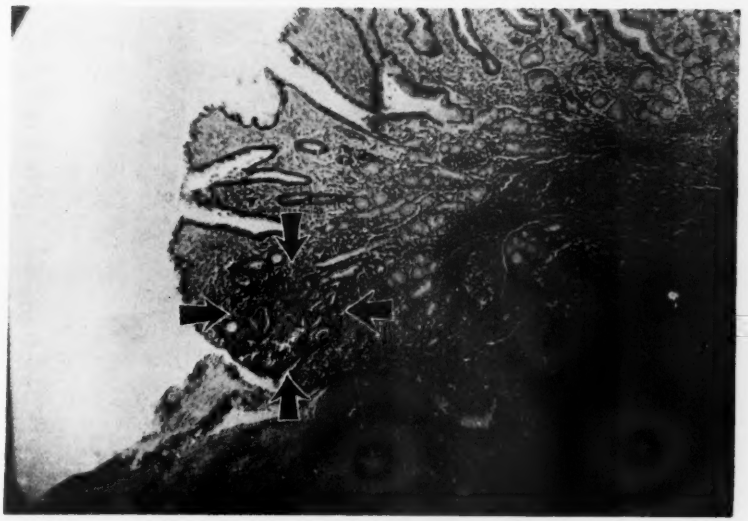


Fig. 3

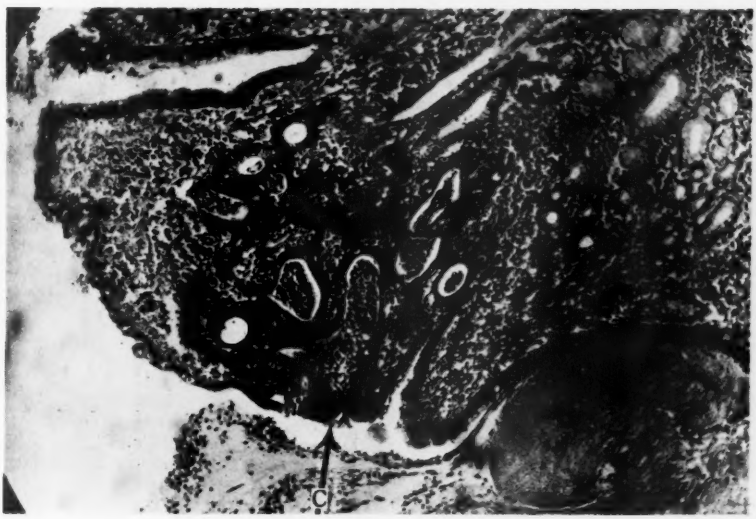


Fig. 4

Fig. 1

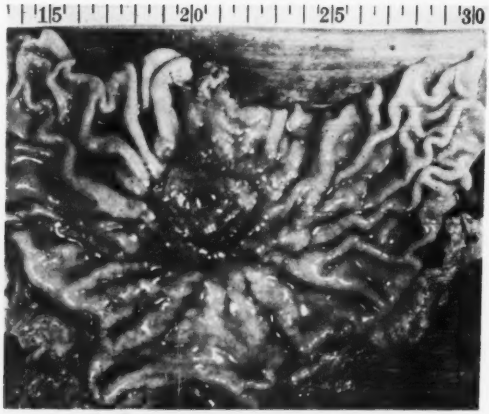


Fig. 2

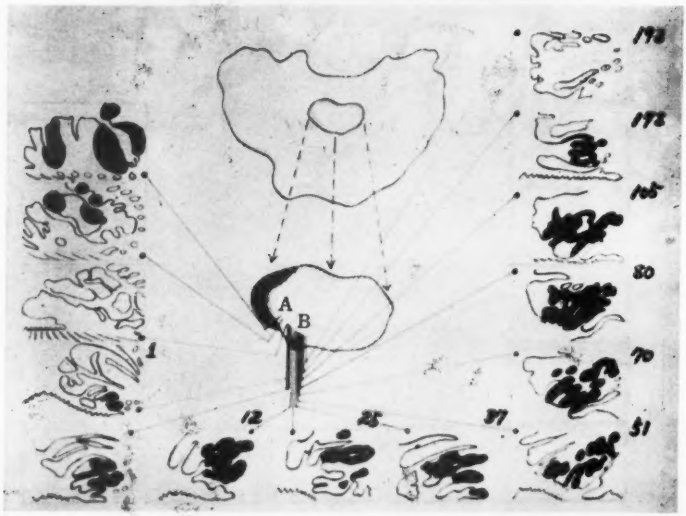
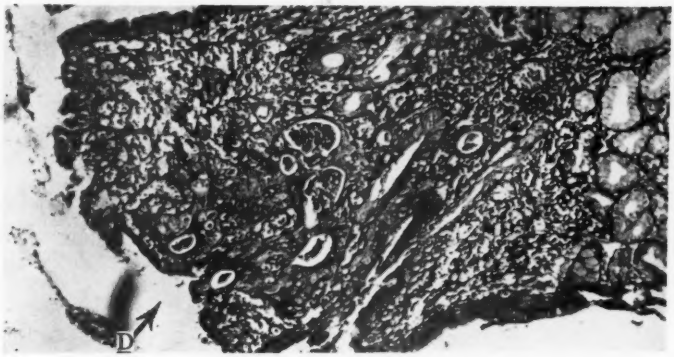


Fig. 5



[GANN, Vol. 44; March, 1953]

THE CYTOLOGICAL EFFECTS OF CHEMICALS ON ASCITES SARCOMAS
II. SELECTIVE DAMAGE TO TUMOR CELLS BY $CaCl_2$, $AlCl_3$ AND H_2O_2[1)]

(With Plate V)

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In the preceding paper of this series, the effects of podophyllin, the mitotic poison, on cell division in the ascites tumors of rats and its influence on the growth of this type of tumor were reported (Makino and Tanaka 1953). It was concluded that suppression of tumor growth and considerable prolongation of the tumor-bearing rat's life occurred as a result of complete damage to most of the tumor cells by the judicious injection of podophyllin. However, some of the tumor cells remained unaffected and became the primary source of renewed malignant groth. Why some of the tumor cells should remain alive and undamaged by the drug is entirely unknown. A possible view to this phenomenon is that in a transformation into the resistant form, the surface of these cells may be altered in some manner, which renders them impermeable to the noxious substance in the surrounding medium. The present investigation was undertaken to confirm the occurrence of this phenomenon and, if possible, to examine the effects of some chemicals which act as a dehydrating agents on cells.

The authors acknowledge with pleasure the valuable advice of Dr. T. Sakamura and his cooperation in supplying chemical agents used here. This work was done in the Zoological Institute of Hokkaido University, Sapporo, Japan, and the manuscript was prepared by the senior author during his stay at the Tissue Culture Laboratory of George Washington University Cancer Clinic, Washington, D. C., U. S. A., through the generosity of Dr. Ivor Cornman, to whom the senior author is greatly obliged.

The chemicals used in these experiments were the dehydrating agents, $CaCl_2$, $AlCl_2$ and H_2O_2. The experiments were carried out with the Yoshida sarcoma and MTK-sarcomas, all being ascites tumors of white rats kept in our laboratory.

1) Contribution No. 280 from the Zoological Institute, Faculty of Science, Hokkaido University, Sapporo. Japan. Supported in part by a financial aid granted from the Scientific Research Fund of the Ministry of Education.

Intraperitoneal injections of these chemical solutions were made in the animals on the 6th day after transplanatation of the tumor. At this time the most active proliferation of the tumor cells is to be obtained in the peritoneal cavity of the host. For observations, smear preparations were made from a droplet of the tumor ascites obtained by abdominal puncture at appropriate intervals following the application of the chemical (for technique, see Makino and Tanaka 1953). In each series of experiments, injections were made in five or six tumor-bearing animals, with the same number of untreated tumor-bearing animals as control. The following observations and discussion were described on the basis of the summarized results from the experimental animals in comparison with those from control animals.

OBSERVATIONS

1. Effect of $CaCl_2$

After several tests employing different concentrations, it was found that a 0.25 M solution of $CaCl_2$ prepared with Ringer's salution exerted a selected effect on tumor cells.[2] Two cc of this solution were injected with a glass pipette into the peritoneal cavity of a 6th-day-tumor animal bearing the Yoshida sarcoma. The observations were based on smear preparations made at various intervals following the injection of $CaCl_2$.

Fifteen to twenty minutes after injection, the tumor ascites displayed a large number of damaged tumor cells (Figs. 4–5). The drug damaged the mitotic somewhat more than resting cells. This solution first exerted its influence on the cytoplasm. The cells were damaged by a breaking down of the cell bodies, followed by a pycnotic disintegration of the naked nuclei, or of the chromosomes after irregular thickening (Fig. 6). The effect of the chemical seems heightened on cells of relatively large size and those showing mitotic abnormalities.

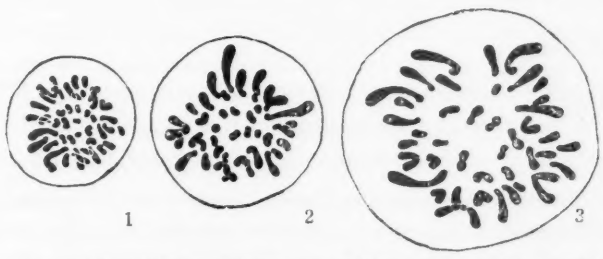
The number of affected cells increased with time following injection. The extent of cellular disintegration was manifested by the amount of cellular debris in the ascites. In the ascites 30 minutes after injection, more than one third the number of tumer cells under observation were disintegrating cells. Forty to fifty minutes after injection, about half the number of the observed cells were found damaged. In the tumor ascites sampled 60 to 70 minutes after injection, most tumor cells were in damaged condition. Rough counts in this stage indicated that over 70 per cent of the tumor cells were undergoing degeneration. By this time there appeared in the tumor ascites a number of eosinophilic leucocytes; they were entirely unaffected by the chemical. It was also found that, interspersed among damaged

2) Preliminary accounts on the effects of this chemical have been given in the paper of Makino, 1952 b.

tumor cells, there were a certain number of tumor cells of small size, which persisted undamaged by the chemical. These were characterized by a small amount of cytoplasm and a single, well-defined uncleus. In the meantime, these small-sized tumor cells began to divide (Fig. 7). The dividing cells increased with time. Three to four hours after injection, the multiplication of these residual cells became a conspicuous phenomenon showing many dividing figures in the tumor ascites. As a result of multiplication of these cells, the tumor renewed growth.

As thus far noted, injection of a sublethal concentration of $CaCl_2$ solution into the peritoneal cavity of tumor-bearing rats resulted in damage to the greater number of tumor cells. Yet, part of the tumor cells remained unaffected by the chemical. The existence of these resistant tumor cells[1] results in a renewed development of the tumor in the treated animals.

Attention should be called on to the nature of the small-sized tumor cells which remain undamaged by the chemical. They divide in an almost regular mitotic manner. The behavior of their chromosomes during division is apparently also regular. Detailed observations were made on the chromosomes of these tumor cells by morphological analysis of the individual chromosomes which revealed that the chromosome complex of these cells is essentially similar to that previously found in the tumor stem (or strain) cells by the present authors (Makino 1952 a, b; Makino and Kano 1953; Makino and Tanaka 1953). The resistant tumor cells



Figs. 1-3. Metaphase complexes of resistant tumor cells, showing sub-diploid chromosomes (±40). From $CaCl_2$ experiments. Fig. 1, dividing cell, 60 minutes after injection of 0.25 M $CaCl_2$. Fig. 2, the same, 3 hours after injection of 0.25 M $CaCl_2$. Fig. 3, dividing cell observed 2 days after injection of the tumor ascites from the animal killed by the injection of 0.5 M $CaCl_2$.

of the Yoshida sarcoma showed at metaphase a characteristic complex of approximately 40 chromosomes, consisting of 22 to 24 rod-shaped chromosomes and 16 to 18 J- and V-shaped ones (Figs. 1-3). On the basis of this finding, there is no doubt that the small-sized, resistant tumor cells, which have persisted without damage by the chemical, are none other than the tumor stem (or strain)

1) Borst (1936) described the existence of the resistant cells in tumor.

cells. The numerical data derived from the study of the material three hours after injection clearly illustrate the above fact by indicating that almost all the dividing cells derive from the tumor stem cells, as evidenced by the presence of approximately 40 chromosomes having their own peculiar characteristics (Table 1, No. 1).

Higher dosages of $CaCl_2$ solution, such as 0.5 M and 0.75 M solutions, produced greater damage to tumor cells, but were lethal to the host animals. For instance, the injection of 0.5 M solution of this chemical killed the animals after about one and half hours. The observation of the tumor ascites from these dead specimens showed a large number of damaged tumor cells and together with them a number of tumor cells at the resting stage characterized by a small amount of cytoplasm and single, compact nuclei, namely, the resistant tumor cells. On examination of the tumor ascites about two hours after death of the host, the degeneration of damaged cells was more pronounced than in the former, but a certain number of the resistant tumor cells still remained free from damage. About 0.3 cc of the tumor ascites taken from these dead specimens about three hours after death, were injected into the peritoneal cavities of new rats. About 48 hours after this injection, many dividing figures were observed in the peritoneal cavities of new hosts. At this time, almost all dividing cells were derived from the tumor stem cells, as indicated by their approximately 40 chromosomes of the characteristic shapes and sizes (Fig. 3). Numerical data illustrating these facts are summarized in Table 1, No. 2.

2. Effects of $AlCl_3$

Experiments were performed with both the Yoshida sarcoma and MTK-sarcoma I as material. These tumors proved to respond in essentially similar manner to the $AlCl_3$ solution, hence the following descriptions apply to both cases. After

Table 1. Results of observations on dividing tumor cells with approximately 40 chromosomes and those showing mitotic abnormalities, after treatment with $CaCl_2$, $AlCl_3$ and H_2O_2 in the ascites sarcomas of rats.

	Chemicals applied	Hrs. after injection	Cells with chrom. numbers of 39-42	Cells with chrom. numbers of 36-38 or 43-46	Cells showing abnormalities
No. 1	0.25 M $CaCl_2$	3 hrs.	89.5%	6.5%	4%
No. 2	0.5 M $CaCl_2$	48 〃	88.5%	10.5%	1%
No. 3	0.125M $AlCl_3$	6 〃	91.0%	4.4%	5.6%
No. 4	2% H_2O_2	10 〃	91.5%	5.0%	4.5%
	〃	12 〃	88.0%	7.0%	5.0%
	〃	15 〃	82.7%	8.1%	10.2%

preliminary tests, it was found that the application of one cc of 0.125 M solution

of $AlCl_3$ prepared with Ringer's solution exerted destructive influence on tumor cells. As in the former case, injection rf the chemical was made in the tumor animals on the 6th day after transplantation of the tumor, and observations were made with smear preparations prepared at intervals after injection of the chemical.

The examination of the slides showed that the $AlCl_3$ solution produced cytological effects essentially similar to those observed in the case of the $CaCl_2$ injection. Following application of this solution, first the cell body broke away and then the disintegration of the naked nucleus followed (Fig. 9).

In tumor ascites taken about one hour after injection, most of the tumor cells were found damaged. After two hours the cell damage was much more pronounced; however, the ascites contained a certain number of tumor cells of small-sized resistant form remained unaffected by this chemical. The unaffected tumor cells began to divide about six hours after injection. An examination of the chromosomes in these dividing cells indicated that the majority of them possessed approximately 40 chromosomes and the characteristic complex peculiar to the stem cells. Numerical data clearly illustrate this feature (Table 1, No. 3). Hence, the renewed growth of the tumor was caused by the proliferation of the resistant tumor stem cells in this case, too.

3. Effects of H_2O_2

The Yoshida sarcoma and MTK-sarcoma displayed a similar response to H_2O_2. The cytological effects of this chemical are apparently the same as those produced by the former two chemicals. The intraperitoneal injection of one cc of 2 per cent H_2O_2 in the tumor-bearing animals induced breaking down of the cell body followed by the pycnotic degeneration of the naked nucleus in tumor cells (Figs. 10–12). As compared with the former two chemicals, this chemical seems to show a more severe action in damaging cells. About one hour after injection, over 70 per cent of tumor cells observed were undergoing degeneration. Two hours after application the damaged cells increased to about 90 per cent of the observed cells. The eosinophilic leucocytes shrank considerably. The neutrophilic leucocytes were actively increasing in number. At this time, there were present in the tumor ascites a certain number of tumor cells of small size, which remained without damage. These unaffected tumor cells began to divide about eight to nine hours after application of the chemical. They divided more actively with time, and the tumor ascites sampled from twelve to fifteen hours after injection contained many dividing cells. Close investigation of the chromosomes in these dividing cells made clear that most of them were the tumor stem cells, because they possessed approximately 40 chromosomes with the characteristic constitution. Numerical data showing this feature are given in Table 1, No. 4. With the passage of time the number of tumor cells increased through multiplication, causing

the renewed growth of the tumor in the treated animal.

It should be added here that the application of H_2O_2 resulted in a prolongation of the life of some treated animals. They lived for about 20 days; namely some 10 days longer than untreated animals.

DISCUSSION

As described above, the cytological effects of $CaCl_2$, $AlCl_3$ and H_2O_2 on the tumor cells of ascites sarcomas of rats are similar for all three substances in the concentration used. There is first a break-down of the cytoplasm followed by disintegration of the resulting naked nucleus. Generally, those showing mitotic abnormalities are damaged first by these chemicals. Those in process of division seem also to be sensitive to the action of the chemicals. Of the three substances tested, H_2O_2 produced the most damage. Furthermore, this chemical was not completely selective for the tumor cells, since some leucocytes were also affected.

After the use of each of the chemicals, it was found that a certain number of tumor stem cells remained unaffected and alive. Though there occurred a temporary reduction of growth of the tumor as a result of the damage to most of the tumor cells, there was no complete recovery from the tumor. Upon cessation of the action of the action of the chemicals, the resistant stem cells began division and caused the renewed growth of the tumor in the treated animals. Complete recovery can be expected only after complete damge to the resistant tumor stem cells. Thus, the general results obtained here are similar to those attained in the previcus study with podophyllin by Makino and Tanaka (1953).

The resistant tumor stem cells are generally characterized by a small amount of cytoplasm and a single, well-defined compact nucleus with distinct nucleoli. They divide with regular mitotic behavior. They possess a characteristic chromosome complex which is specific to the kind of tumor (see Makino 1952 a, b; Makino and Kano 1953). The individuality of the chromosomes in the tumor stem cells remains unchanged during successive transplant generations. The continuity and the growth of the tumor during successive transplant generations are primarily due to the presence and protiferation of these stem cells.

The question why a few of the tumor stem cells should remain undamaged by the chemicals applied, whereas the majority of tumor cells are damaged, is one of the important subjects of investigation in cancer therapy. It is highly probable that, when exposed to injurious conditions, some tumor stem cells are able to protect themselves by transforming into the resistant form. Possibly, this transformation may be accomplished by the cells becoming impermeable to the noxious substance in the surrounding medium. In other words, the resistant nature of the stem cells may result from a phsiological change or changes in the cell membrane.

The former study with the application of a mitotic poison, podophyllin (Makino and Tanaka 1953), and the present investigation with dehydrating agents, such as $CaCl_2$, $AlCl_3$ and H_2O_2, have shown that the resistant tumor stem cells of ascites tumors of rats can not be completely damaged with doses of the drugs not lethal to the hosts. Further, the resistant tumor cells can live for a time after the death of the host. It is these tumor cells that cause the renewed growth of the tumor in the treated animals. The physiological nature of these stem cells remain entirely unknown. The necessity arises thus, to investigate the general physico-chemical properties of these tumor cells, in particular such properties as permeability, excitability phenomena, metabolism and adsorption effects.

SUMMARY

Two cc of 0.25 M $CaCl_2$ solution, 1 cc of 0.125 M $AlCl_3$ solution, or 1 cc of 2 per cent H_2O_2 were injected into the peritoneal cavity of the tumor-bearing rats of ascites sarcomas, and the effects of these chemicals were investigaled with smear preparations made at various intervals after injection of the chemicals. It was found that these chemical first exerted their influence on the cytoplasm, invariably inducing the breaking down of the cell body of the tumor cell. Then the pycnotic disintegration of the resulting naked nuclei followed. There occurred a temporary retardation of growth of the tumor to greater or less degree in every experimental animals as a result of damage to the majority of the tumor cells by the application of these chemicals, but a certain number of the resistant tumor cells always remained unaffected by the chemicals applied, so long as the dosage employed was sublethal to the host. Upon cessation of the action of the chemicals, these resistant tumor cells began multiplication and caused the renewed growth of the tumor in the treated animals. There is a great need to study the physico-chemical properties of these resistant tumor stem cells in connection with cancer chemotherapy.

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EXPLANATION OF PLATE V

All are photomicropgraphs. Figs. 4-7, from $CaCl_2$ experiments. Fig. 4, destruction of tumor cells. 30 minutes after injection of 0.25 M $CaCl_2$. 400×. Fig. 5, the same, 60 minutes after iejection. Tumor cells of small size have remained unaffected (indicated by arrows). 400×. Fig. 6, abnormal condensation of chromosomes in a tumor cell, observed 60 minutes after injection of 0.25 M $CaCl_2$. 1000×. Fig. 7, dividing cell showing subdiploid chromosomes, observed 10 hours injection of 0.25 M $CaCl_2$. 600×. Fig. 8, tumor cells just before injection of $CaCl_2$. From an animal on the 6th day after transplantation of the tumor. (Control). 400×. Fig. 9, destruction of tumor cells. 1 hour after injection of $AlCl_3$ (0.125 M). 800×. Figs. 10-12, from H_2O_2 experiments. 1000×. Figs. 10, breaking down of the cytoplasm remaining the nuclei naked. About 2 hours after injection of 2% H_2O_2. Fig. 11, irregular thickening of chromosomes occurring in a naked tumor cell. 2 hours after injection. Fig. 12, disintegration of chromosomes in a tumor cell. 2 hours after injection. Figs. 4-8, from Yoshida sarcoma. Figs. 9-12, from MTK-sarcoma I.

要　旨

薬品の白鼠腹水肉腫に及ぼす細胞学的影響（第二報）

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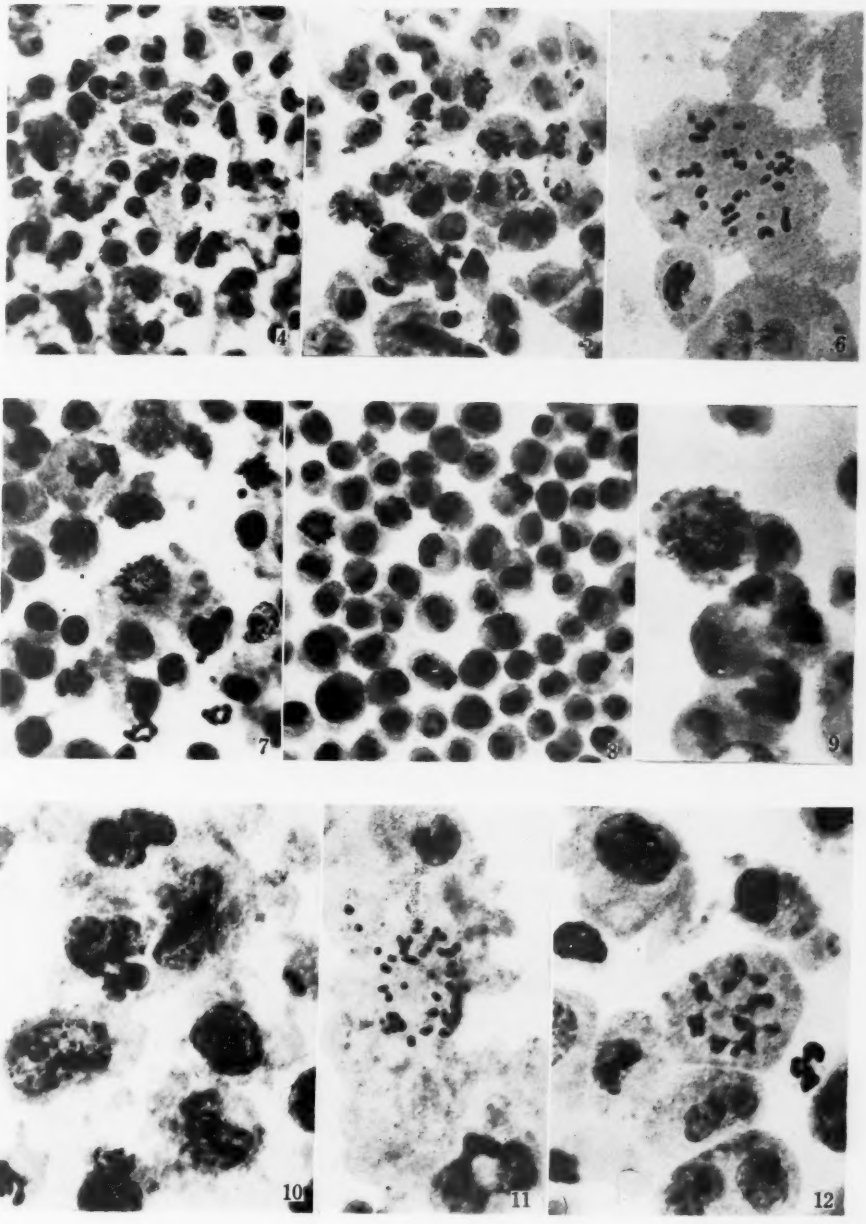
（北海道大学理学部動物学教室）

2 cc の 0.25 M $CaCl_2$, 1 cc の 0.125 M $AlCl_3$ 及び 2% H_2O_2 を，移植 6 日目の吉田肉腫ならびに MTK 肉腫ネズミの腹腔に注射して，腫瘍細胞への影響を細胞学的に観察した。この3種の化学薬品は例外なく，まず腫瘍細胞の細胞質を破壊し，ついで裸出した核が退化を起す，大部分の腫瘍細胞が破壊されるので，肉腫の成長はここで一時的に退行するが，いずれの場合においても，一群の母系細胞は薬品によつて破壊されるとなく生きのびるので，やがてそれらの分裂増殖によつて，再び腹水肉腫が形成される。如何なる機構によつて母系細胞のある者が薬品の破壊をまぬかれるか，これは癌の化学療法においてまず第一に研究しなければならぬ問題である。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[GANN, Vol. 44, March, 1953]

ON CANCERS DEVELOPED UPON ULCERATIVE LESIONS OF THE STOMACH; A STUDY OF THE REGENERATION OF THE MUCOUS MEMBRANE OF THE STOMACH WITH SPECIAL REFERENCE TO ITS MALIGNANT TRANSFORMATION*

(With Plates VI–XIV)

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From the Surgical Clinic of Kanazawa University School of Medicine

I. INTRODUCTION

In individual cases of the gastric ulcer, the extent of the defect of the gastric wall varies greatly not only as to the diameter but also as to the depth. It is quite reasonable either from the clinical view or from the pathological to distinguish the penetrating ulcer with a marked gap in the main muscle coat from the superficial one without it.

The superficial ulcer can be converted of course into the deep one by progressive sloughing due to the peptic process, but, if not large, heals not infrequently with appropriate treatment, the defect in the mucous membrane being repaired by regeneration. In case of deep ulcer with marked gap of the muscle coat, the chance of healing seems to be incomparably scanty. (In Fig. 1 is shown an example of such cases). Beside the width of the surface to be covered the existence of organic change in the blood vessel (endarteritis) may play an important rôle for the retardation of healing in such a case. In contrast to the fact that the chance of witnessing the healing of deep ulcer is extremely rare, the opportunity of discovering the incipient stage of the ulcer-cancer is obviously increasing in parallel to the frequency of gastrectomy. I want to demonstrate here several typical specimens out of my 20 years' surgical experience concerning the carcinomatous development upon ulcerative lesions of the stomach.

II. ULCER-CANCER SATISFYING THE CRITERIA OF HAUSER[5][6]

The essential of Hauser's criteria concerning the determination of an ulcer-cancer consists in:

1) Partial carcinomatous infiltration of the edge or the base of the ulcer;

* Demonstrated before the Assembly of the West Section of the Japanese Pathological Society held in Kanazawa on 10th Oct., 1952.

2) complete destruction of muscular coat and its replacement by fibrous tissue; and

3) fusion of the muscularis mucosae and the muscular coat at the margin of the ulcer.

Since the superficial ulcer devoid of a gap in the muscular coat can also be transformed into malignancy and in all cases of ulcer-cancer more or less later the whole lesion can be invaded by tumor cells, the tenability of these criteria for the determination, whether the cancer in question has arisen in the gastric ulcer or not, is extremely doubtful. Though it may be true that the majority of gastric cancers which do satisfy his criteria have arisen in chronic ulcer, the fact can by no means exclude the possibility, that those which does not, also may have arisen in it. It should be emphasized, therefore, that *Hauser's criteria are briefly helpful for the discovery of the initial stage of a certain category of ulcer-cancers.*

In Fig. 2 is demonstrated an incipient cancer, so small as a grain of millet, found beneath the edge of the muscularis mucosae on one side of a deep ulcer. The lesion, 6.0×3.5 cm in size, showed an appearance of a large callous ulcer, and the mucosa distant from it were thrown into folds. Histologically, too, apart from the strictly localized invasion of the cancer, the specimen presented all the possible characteristics of the callous ulcer, such as marked gap in the main muscle layer, the over-hanging edge of the muscularis mucosae, the dense scar tissue in the base of the ulcer, the existence of endarteritis and so forth. And the mucosa remote from the lesion was redundant. In this case, notwithstanding the smallness of the focus, no one hesitate to diagnose it a cancer, for beside the conspicuous anaplasia in cells the heterotopic growth is quite explicit.

Several analogous specimens can be demonstrated. In all of them the grade of malignancy of the tumor cells is fairly advanced (some of them undergoing gelatinous degeneration), in spite of the localized site in the submucosa beneath the edge of the muscularis mucosae. This characteristic finding is not well comprehensible, without taking into consideration the pre-existing circumstances, namely, the undermining growth of the regenerated mucosa. In most of the large callous ulcer, the edge of the muscularis mucosae is prone to curl around and to hang over the edge of the submucous and main muscle layers which are more intensely eroded, as if to offer a favourable condition for the undermining growth of regenerated mucosa. In Fig. 3 is shown the heterotopic development of the regenerated mucosa as seems to be the sequel of such an incident. Newcomb has called attention to the frequent appearance of such heterotopic growth of the regenerated mucosa, but he regarded it rather as harmless occurrence.

Corresponding to the biological demand the regenerative tissue is provided with ability rapidly to grow in opposition to unfavourable circumstances; an ability

certainly resembling the property of malignant new-growths. The morphological expression of this biological ability consists in heterotopia and anaplasia. The hyperchoromatism of the nucleus, lack of polarity and increased appearance of mitotic figures belong to the latter and the direct mucous covering of the muscle layer or the scar without interposition of muscularis mucosae may pertain to the former. The only characteristic which distinguishes the regenerated epithelium with marked anaplasia from the true malignancy consists in the presence of reversibility. In case of the normal regeneration the anaplastic aspect disappears as soon as the reparation is completed. But if the unfavourable circumstances for the reparation last, as in case of the deep callous ulcer, the oppotunity for malignant transformation can be furnished.

III. Cancerisasion in Situ of the Mucosa Bordering an Ulcer.

Above, I treated of a case, in which the undermining growth of the regenerated mucosa seems to precede the acquisition of permanent anaplasia. In such a case the initial focus of cancerous development takes place beneath the edge of the muscularis mucosae, a condition, which obviously facilitates the histological diagnosis of cancer even in its most initial stage. No wonder that the most of the description concerning ulcer-cancer correspond to this category (Newcomb[12], Konjetzny[7], in detail consult Borrmann[1]). However, if the acquisition of permanent anaplasia *in situ* takes place, the primary site of malignant transformation should be found within the mucosa upon the margin of the ulcer. In Fig. 4 we see a group of extraordinary cells occupying the superficial layer of the mucosa upon the margin of an ulcer. The constituent cells are unequal in size, hyperchromatic, and irregularly arranged. In certain parts they are devoid of basement membrane forming solid aggregations (Fig. 5). Cytologically they can be hardly distinguished from cancer cells, though their location is absolutely confined to the mucous membrane.

Since in this case the invasive growth cannot be detected, and, according to the classical opinion of pathologists it should not be comprised in the category of the incipient cancer. An analogous but certainly more advanced case with invasion into the submucous layer was described in detail by Moskowicz.[10] And since the establishment of the concept of "cancer in situ "(Broders[2]), those cases with strictly localized focus within the mucosa became the object of profound interest of pathologists, and quite analogous cases were reported by Mallory,[8] Guttman et al,[4] Konjetzny,[7] Murakami,[11] etc.

IV. Sequel of the Incipient Ulcer-Cancer.

In either case, once a focus of cancer is formed, it extends first circumferentially

inside the loose submucosa bordering the ulcer and then towards the scar covering the base of the ulcer. Opposite to the generally adopted opinion, I believe in the non-resistance of the scar to the invasion of tumor cells owing to the absence of elastic fibres. In Fig. 6 is demonstrated a specimen in which the infiltration of cancer has exceeded the submucosa and has widely occupied one side of the ulcer. In Fig. 7 the invasion has further advanced; the border of the ulcer is infiltrated circumferentially, leaving the floor unaffected. Here I do not enter into the question of whether in such cases the initial focus be single or not. The extent of cancer infiltration is shown in Fig. 8, still broader, attaining the floor of it, yet the major part of the floor is free from invasion. In Fig. 9 the floor too is completely invaded, but the direction of infiltration is surmisable with the arrangement of tumor cells, namely, the primary focus, composed of gelatinous cells, is seen beneath the muscularis mucosae at the margin of the ulcer whence the invasion radiates to the floor. In next case the carcinomatous infiltration is most advanced. Though in this case the lesion was so large that total gastrectomy was necessary, the vestige of the callous ulcer is traced fairly well, such as the gap of the muscle coat and the exposure of its edge on the floor of the exulceration, the overhanging edge of the muscularis mucosae, the existence of the scar at the base of the ulcer, though completely infiltrated by cancer, and so forth. With elastic fibre stain the circumstances are revealed most explicitly (Fig. 10).

Evidently the last 2 cases no longer satisfy Hauser's criteria, and it is usually regarded as unreasonable to comprise these cases into the category of ulcer-cancer. Still the preservation of the characteristic of the chronic ulcer in these specimens convinces us their development upon the ulcer, for most of secondarily exulcerated cancers of other origin are provided with quite different features. For, in examples in case of the exulceration of massive, proliferating cancers the edge of muscularis mucosae shows upturn figure towards the cavity (Büchner)[3], and in those of exulceration of the diffusely infiltrative cancers the muscularis mucosae shows a parallel course to the surface of the main muscular coat. In both cases the course of muscle coat can be traced, even if it may be excessively invaded by cancer.

V. Malignant Transformation of the Regenerated Mucosa Covering a Superficial Ulcer

Another category of gastric cancer in relation to the ulcerative process of the stomach consists in the malignant transformation of the regenerated mucosa covering the shallow superficial ulcers, which presents a fairly different aspect. Most of the small superficial necrosis of the mucosa not affecting the muscularis mucosae subsequent to the chronic gastritis heals without leaving any marked

deformity in the mucous membrane. In such cases though no marked defect in the mucosa can be detected with naked eyes, a number of small shallow dents covered with thin mucous layer are found under the microscope. Taking into consideration the experimental work of Mann,[9] the latter should be interpreted as the healing of the former. I have a case, which can be explained as the sequel of such an instance. In this case a shallow hollow covered with thin layer of mucosa was found near the pyloric ring, which was bordered by a low margin. In the microscopical examination of this part, there was found a small defect on the mucosa, in the middle of which existed a small focus of cancer, 0.5 cm in diameter, which had already invaded into the submucous layer.

Carcinomatous transformation of the regenerated mucosa in case of the superficial ulcer is more easily traced when the lesion is large. In Fig. 11 is demonstrated a gastric cancer of such a kind in its most incipient stage. This is a case of typical leather-bottle stomach. In this case, in spite of a wide lesion in the mucosa, the erosive process did not surpass the muscularis mucosae and there is absolutely no defect in the main muscle coat. The leather-bottle appearance was caused by an excessive fibrous overgrowth in the gastric wall beneath the muscularis mucosae. Fluoroscopic findings as well as the absence of hydrochloric acid in the gastric juice have lead us to diagnose this case gastric cancer, and even with the inspection of the specimen and its cut surface least doubt was raised as to its malignant nature. Nevertheless, the prevailing findings under the microscope were of inflammatory nature, and confined to a strictly limited area of the mucosa on the margin of the ulcer, an extremely small focus of cancer, scarcely attaining the size of a millet (diameter 0.7 cm) was found (Fig. 12). Obviously it deals with a cancerisation in situ, for the muscularis mucosae is not yet penetrated. Still, as shown in Fig. 13, the grade of malignancy is fairly advanced; mitosis is abundant, the basement membrane is scarcely observable and the arrangement of cells is quite irregular, so that the observation under the high power magnification leaves least question for its malignant nature.

The pronounced tendency to malignant transformation of such regenerative mucosa at the margin of large superficial ulcers was especially emphasized by Konjetzny.[7] Following observations may sustain his view. In Fig. 14 is shown the regenerative mucosa found on the floor of a shallow ulcer. In this case, though the atypical epithelial proliferation is fairly marked, the infiltrative growth is absent. In the next case, in which a large cancer existed in the pyloric region, was found a superficial ulcer apart from this cancer. There was no gap of muscle coat in this ulcer and the floor of it was covered by regenerative mucosa (Fig. 15). The irregularity of cells in this part is more conspicuous than in the previous case and the submucous tissue is already invaded. In this case a small cancer is developing upon the superficial ulcer independently of principal focus

in the pyloric region.

From these observations following conclusion can be drawn. Assume here the the existence of a superficial ulcer and its healing by covering of regenerated mucosa; if in such a stomach another process in favour of the cancer formation (for example atrophic gastritis) develops, the part most intensively inclined to malignant transformation is none other than this regenerated mucosa.

As above mentioned, the regeneration itself is a vital function provided with more or less marked anaplasia of cells, and, if the preceding defect of tissue surpasses a certain extent, more or less intensive heterotopia follows. The latter promotes the former, while the former, as a matter of course, accelerates the latter in its turn.

VI. Malignant Transformation of the Regenerated Mucosa Covering the Floor of a Deep Ulcer

It is hitherto rather neglected to take into consideration the behavior of the floor of the deep ulcer, for, as I have related at the commencement of this paper, the deep ulcer heals but rarely with covering of regenerated mucosa. The following experience, however, may suggest the significance of the base of ulcer in regard to the cancerisation.

It concerns a case of a large callous ulcer in pyloric region (Fig. 16), in which an islet of regenerated mucosa was fixed upon the floor of the ulcer. In this stomach a condition favourable for the growth of cancer seems to have ripened, and consequently two different foci of cancerisation have developed within a single ulcer. The one, modeling after the cancerisatio in situ at the margin of the mucosa, is situated upon the edge of muscularis mucosae (Fig. 17), being composed of sphaerical cells undergoing gelatinous degeneration (Fig. 18). The tubular structure is already lost in its superficial part (ca. simplex). The other focus, affecting the cancerisation in case of a shallow ulcer, is situated in the islet of regenerated mucosa upon the floor of the ulcer (Fig. 19). It is tubular in structure and the constituent cells are cylindrical (ca. adenomatodes). The edge of the muscle layer beneath it is already invaded by it (Fig. 20).

This experience implies that the regenerated mucosa covering the floor of the deep ulcer is also prone to the malignant transformation.

VII. Cancerous Development in the Base of a Penetrating Ulcer formed by the Glandular Organ outside the Stomach

It concerns again the cancerisation in the base of the deep penetrating ulcer, but the soil is another glandular organ outside the stomach. As example of such an instance the following experience should be described. In this case the base

of a large penetrating ulcer on the lesser curvature was covered by the left lobe of liver, so that the wide gastrectomy combined with resection of the liver was necessary (Fig. 21). On the margin of this large ulcer, in spite of the certain irregularity in form of cells, the cancerous transformation did not yet take place, while in that part of liver, with which the floor of the ulcer was furnished, was found a focus of cancer with histological feature of cholangic carcinoma (Fig. 22). Since the site of this new-growth is strictly localized in the small superficial part of the liver corresponding to the floor of the ulcer, the cancerisation in this case should be related more to the circumstances inside the stomach than to those in the liver. Hitherto I was rather inclined to regard the floor of the ulcer as more innocent than the margin as to the initial focus of cancer; and, if the margin of ulcer is free from cancerisation, I usually abstained from reckless removal of the base of the ulcer. But, as in this case, if the base is formed by other epithelial organs, certain precautions should be paid for the possibility of cancer development in it. This may be of significance from the surgical point of view, but I shall not enter into this problem here.

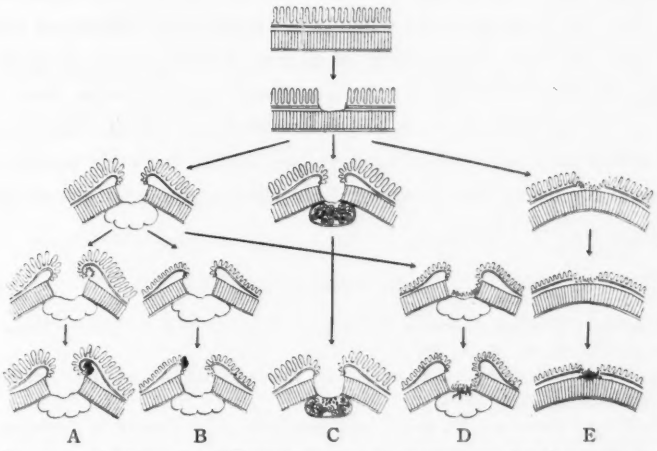

Diagram showing the different modes of cancerous development subsequent to a ulcerative process in the stomach. Focus of cancer is drawn black in the figures of the lower-most row.

A. Initial focus beneath the edge of the muscularis mucosae of a deep ulcer.

B. Cancerisatio in situ in the mucosa bordering a deep ulcer.

C. Cancerous development in the base of a penetrating ulcer formed by the glandular organ outside the stomach.

D. Malignant transformation of the regenerated mucosa covering the floor of a deep ulcer.

E. Malignant transformation of the regenerated mucosa covering a superficial ulcer.

VIII. Conclusions

1. As to the mode of cancerous development in and around the gastric ulcer at least 5 different categories can be distinguished (compare the diagram).

A The establishment of initial cancerous focus beneath the edge of muscularis mucosae of the deep ulcer.

B Cancerisatio in situ in the mucosa bordering the callous ulcer.

C Malignant transformation of the regenerated mucosa covering the superficial ulcer.

D Malignant transformation of the regenerated mucosa covering the floor of the deep ulcer.

E Cancerous development in the base of the penetrating ulcer formed by the glandular organ outside the stomach.

2. In the advanced stage of gastric cancers, too, if they are related to the callous ulcers, the vestige of the latter is well detectable in most of the cases.

3. The regenerated mucosa seems to be more intensively inclined to the malignant transformation than normal mucosa, which fact may deserve notice of clinicians. For, the gastric ulcer, when it is callous and difficult of cure, offers a soil favourable for the development of cancer, and also when it is shallow and healed, seems to be more prone to the malignant transformation than any other part of the gastric mucosa in case of the establishment of the cancerogenic condition. In either case, once an ulcer is formed in the stomach, special attention should be paid for its further progress from the standpoint of the prophylaxis of cancer.

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抄　　錄

胃における潰瘍性病変の上に発生せる胃癌について，前癌性変化を中心として見た胃粘膜の再生

久留　　勝

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1. 胃潰瘍の中あるいは周囲から癌の発生を見る機構には，少くとも次の五つの異つた範疇を区別する事が出来る。

A. 初期病竈の発生が深い潰瘍辺縁の粘膜筋層の断端の下に見られる場合，

B. 深い潰瘍の辺縁粘膜における Cancerisatio in situ,

C. 表在性潰瘍を覆う再生粘膜における悪性変化，

D. 深い潰瘍の底を覆う再生粘膜における悪性変化，

E. 他の上皮性器官によつて作られている穿通性潰瘍の底の癌化。

2. 深い胼胝性潰瘍に関連を持つ胃癌では，進行せる状態においても，大多数の場合その母地のおもかげを証明する事が出来る。

3. 再生粘膜は正常粘膜よりも癌化に傾き易い。この事実は臨床家の注意を要する。何となれば胃潰瘍は胼胝性で治癒の傾向に乏しい場合癌の好発母地をなすのみならず，浅くして治癒する場合も，胃に発癌条件の具備せられる時には，他の胃粘膜よりも悪性化を起し易いからである。すなわち一旦胃に潰瘍の証明せられた場合は，癌の予防の見地から，その経過に特別の注意が払わるべきである。

Fig. 1. Healing of a deep callous ulcer with covering of the regenerated mucosa, which conceals certain irregularity in its tubular arrangement. Note the marked gap of the muscle layer, dense fibrous tissue forming the base of the ulcer and the endarteritis in the artery.

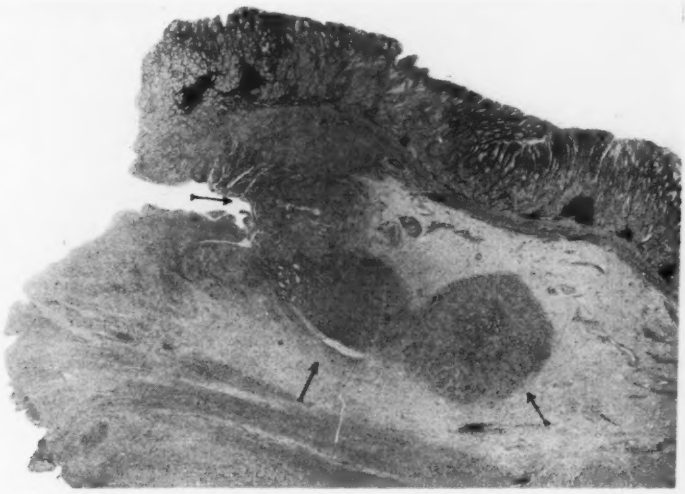

Fig. 2. An incipient focus of cancer (shown with arrow) beneath the edge of the muscularis mucosae at the margin of a deep callous ulcer.

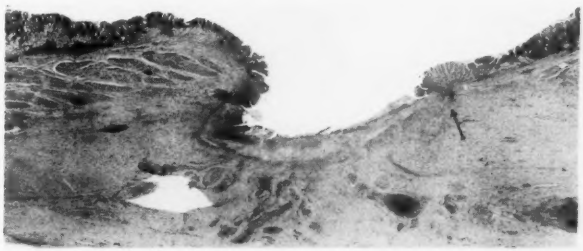

Fig. 3. Heterotopic growth of regenerated mucosa at the margin of a deep ulcer.

Fig. 4. Cancerisatio in situ at the margin of a large callous ulcer. Compare Fig. 5.

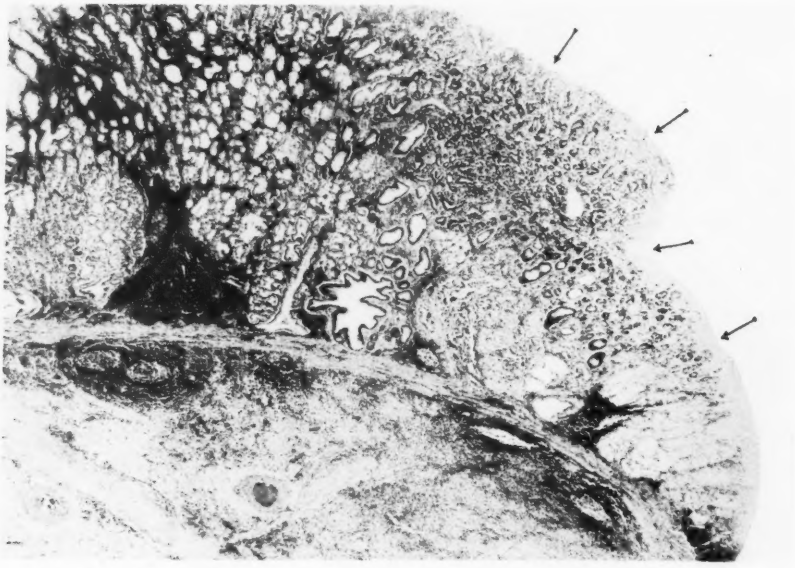

Fig. 5. Enlargement of the part inside the rectangle of Fig. 4.

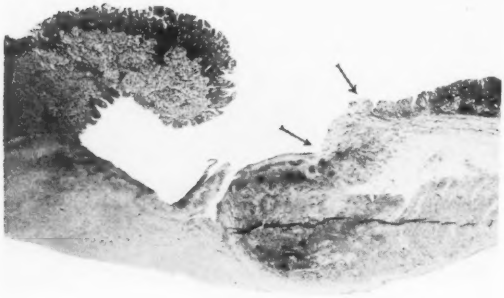

Fig. 6. A deep callous ulcer upon the pyloric ring. The gastric half is mostly invaded by the cancer, while the duodenal half is free from invasion.

Fig. 7. The margin of a large callous ulcer is circumferentially occupied by the cancer (indicated with single arrow), leaving the base of it untouched. The latter is covered by pancreas (indicated with double arrow).

Fig. 8. Carcinomatous development originated at the margin of the ulcer has partially affected the floor of it. The major part of the latter remains still uninvaded.

Fig. 9. The margin of a large exulcerated gastric cancer. The supposed initial focus (c) beneath the edge of the muscularis mucosae has undergone the gelatinous degeneration, whence the carcinomatous infiltration radiates.

Fig. 10. A large exulcerated cancer of the stomach (case of total gastrectomy). The vestige of callous ulcer, such as the overhanging edge of the muscularis mucosae, the gap of the main muscle coat and the scar forming the base of the ulcer, is still well recognizable. Elastica-stain.

Fig. 11. Gross appearance of the leather-bottle stomach accompanied with a large superficial ulcer. The part of cancerisatio in situ is marked with arrow. Compare Figs. 12 and 13.

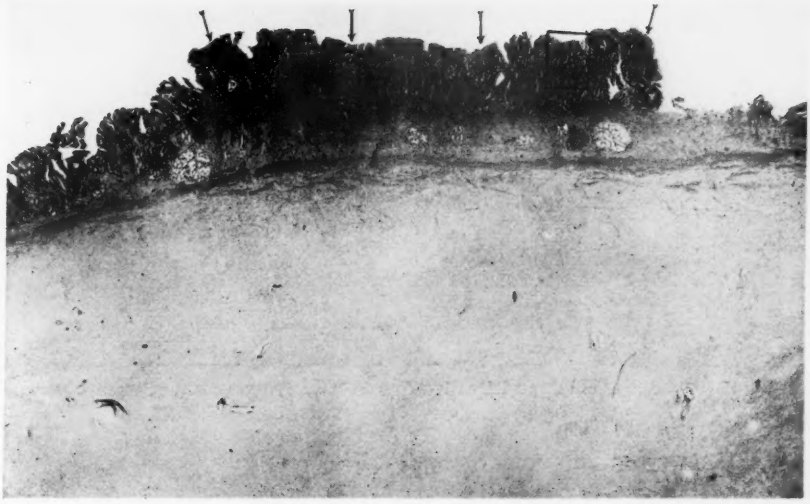

Fig. 12. Millet-sized cancer in situ (marked with arrow) found on the margin of a large superficial ulcer. Section of the part marked in Fig. 11. Muscularis mucosae is not yet penetrated. Excessive fibrous overgowth in the submucosa. The main muscular layer is scarcely visible in the lower-most part of the figure. Compare Fig. 13.

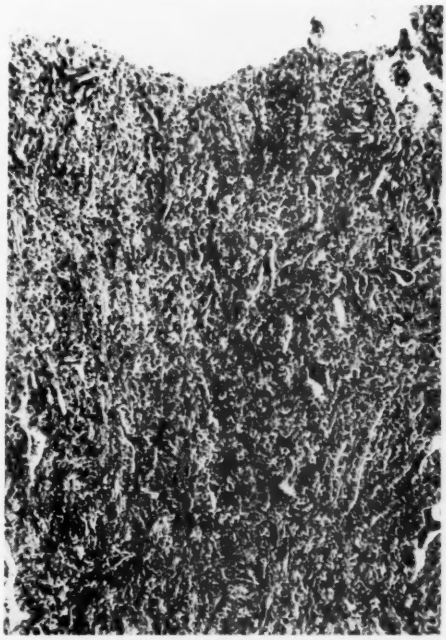

Fig. 13. Enlargement of the part inside the rectangle of Fig. 12.

Fig. 14. Atypical epithelial proliferation found in the regenerated mucosa covering a large ulcer.

Fig. 15. Beginning infiltrative growth of malignantly transformed regenerated mucosa covering a superficial ulcer. Apart from this a large carcinomatous lesion was found in the pyloric region of the same specimen.

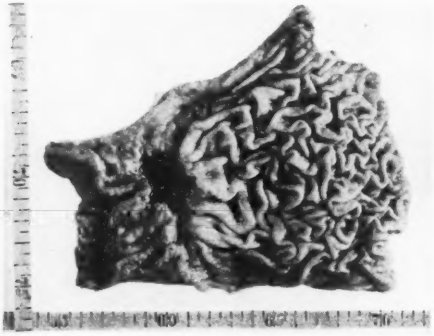

Fig. 16. A deep callous ulcer of the pyloric region, in which 2 foci of cancerisation with different histological feature were found. Compare Figs. 17—20.

Fig. 17. The carcinomatous transformation of the regenerated mucosa at the margin of the ulcer shown in Fig. 16. Compare Fig. 18.

Fig. 18. Enlargement of the part inside the rectangle of Fig. 17.

Fig. 19. A carcinomatous focus (marked with double arrow) found in the regenerated mucosa (marked with single arrow) fixed upon the floor of the ulcer shown in Fig. 16. Compare Fig. 20.

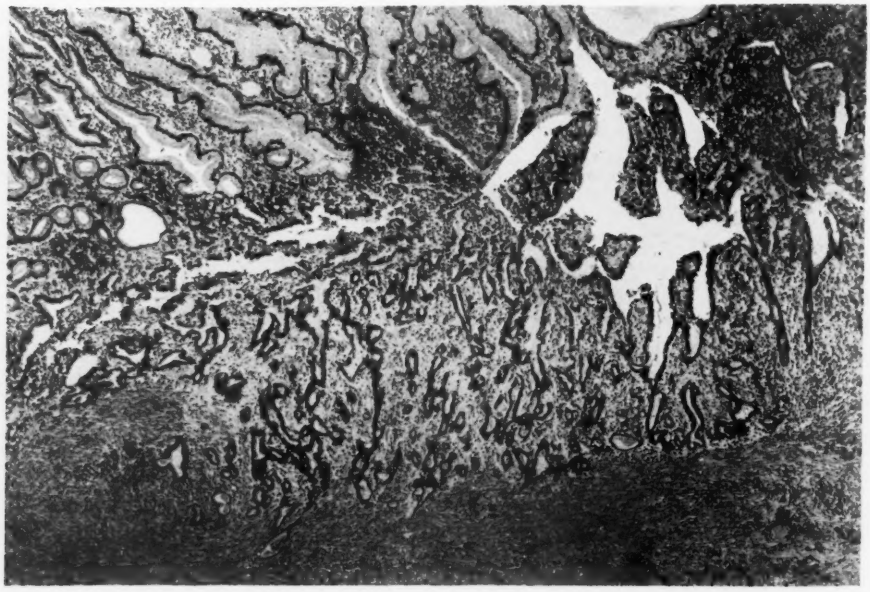

Fig. 20. Enlargement of the part inside the rectangle of Fig. 19.

Fig. 21. Cholangic carcinoma developed in a small superficial part of the liver which has formed the base of a large penetrating ulcer. In the mucosa of the stomach itself no cancerous transformation was found. Compare Fig. 22.

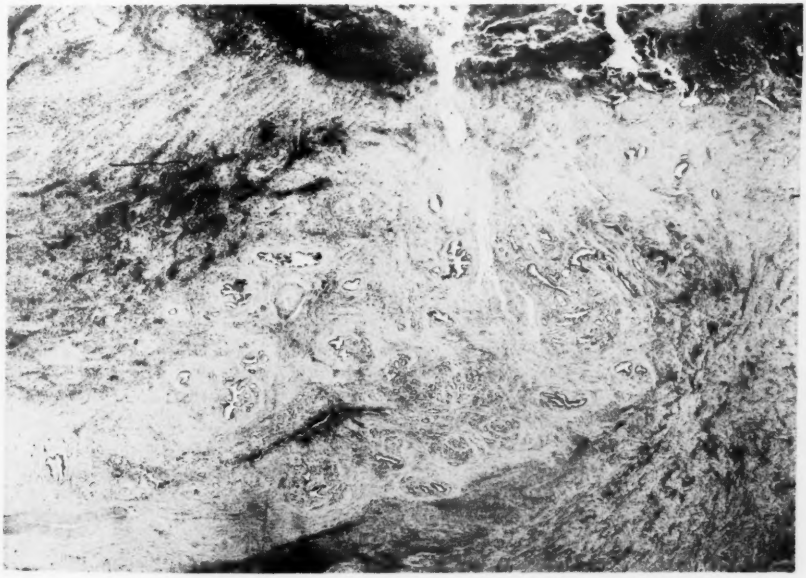

Fig. 22. Enlargement of the part inside the rectangle of Fig. 21.

ANNOUNCEMENTS

Classification of Carcinoma of the Uterine Cervix
Japanese modification to the American plan

In view of the facts that (1) the classification adopted by the International and Fourth American Congress of Obstetrics and Gynecology, 1950, is applicable exclusively to the Radiotherapy, that (2) the Carcinoma of the Uterine Cervix is treated in Japan by two methods, namely by operation and radiation, and that (3) more than one-third of the total cases is operated, the Committee of Carcinoma of the Uterus in the Japanese Obstetrical and Gynecological Society decided to propose the following modification (underlined) in Stages II and III in regard to the vaginal involvement and to apply it for all cases, whether operative or radiological, to be treated in Japan.

Stage 0: Carcinoma in situ.....also known as preinvasive carcinoma, intra-epithelial carcinoma and similar conditions.

Stage I: The carcinoma is strictly confined to the cervix.

Stage II: The carcinoma extends beyond the cervix, but has not reached the pelvic wall. **The carcinoma involves the vaginal fornix.**

Stage III: The carcinoma has reached the pelvic wall. (On rectal examination no "cancer-free" space is found between the tumor and the pelvic wall.) **The carcinoma involves the upper third of the vagina.**

Stage IV: The carcinoma involves the bladder or the rectum, or both, or has extended beyond the limits previously described.

The Committee of Carcinoma of the Uterus, The Japanese Obstetrical and Gynecological Society, Tokyo.

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T. Hasegawa (Tokyo)
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E. Nakayama (Niigata)
H. Yagi (Okayama), Chairman of the Committee.

Japanese League of the Annual Report of Carcinoma of the Uterine Cervix

The Committee has accepted already applications of 22 hospitals throughout Japan to adopt this new modified classification from the year 1953 and to report the results of treatment after 5 years, whether radiation or operation, from 1959. The Japanese Gynecologists may thus join before long the International Committee on this subject.

Address of Chairman;
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